ASPECTS OF THE BIOLOGY AND ECOLOGY OF THE NUDIBRANCH MOLLUSC 'AEOLIDIA PAPILLOSA (L.)'

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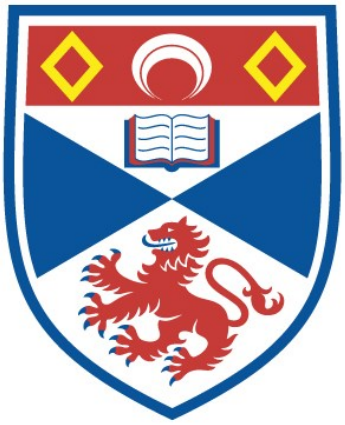
A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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Aspects of the biology and ecology of the nudibranch mollusc *Aeolidia papillosa* (L.)

by

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being a thesis submitted to the University of St Andrews in canditature for the degree of Doctor of Philosophy.

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October 1983.

Declaration.

I declare that the work reported in this thesis is my own and has not been submitted for any other degree. Due acknowledgement has been given for any assistance recieved.

Supervisors Certificate

I certify that Stephen John Hall has fulfilled the conditions laid down under Ordinance General Number 12 and Resolution of the University Court 1967, Number 1, of the University of St Andrews and is accordingly qualified to submit this thesis for the degree of Doctor of Philosophy.

Curriculum vitae

I graduated from U.C.N.W. (Bangor) in 1979 with a B.Sc in Marine Biology and Biochemistry. The work described in this thesis was carried out between October, 1980 and September, 1983.

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To Liz,

ABSTRACT

In this study particular attention has been paid to the foraging biology and ecology of Aeolidia papillosa (L.); an anemone-eating nudibranch mollusc.

'Prey-value', derived in its current biological sense from optimal foraging models, is expected to be an important determinant of prey-selection behaviour. In the present study 'prey-values', or more specifically, food or tissue-values (because intact anemones were not used in the analyses), have been investigated for a range of anemone species. Analysis of the biochemical composition of anemone tissues and their consumption and assimilation by nudibranchs did not reveal any marked interspecific differences. S.troglodytes, however, is indicated as being somewhat different from the other species studied, possibly being assimilated more efficiently and consumed at a faster rate. These factors, in conjunction with the strong preferences shown for S.troglodytes in switching experiments and the apparent preponderance of field associations with this species, do indicate that S.troglodytes may be a more valuable food item for A.papillosa. Analysis of the composite estimates of fitness (growth and reproduction), however, did not reveal any contrasts in performance which could be related to diet. This was almost certainly a result of the marked variability in the performance of nudibranchs within each diet group obscuring any dietary effects which may have obtained.

In the latter part of this study a series of behavioural experiments were conducted which investigated specific aspects of prey-selection behaviour. Using a variety of multiple-prey species choice experiments, data have been collected which show the effects of previous dietary experience on prey-species selection The results of these experiments suggest that at least some of the contradictions in previous reports of prey-species preference by A.papillosa may be accounted for by "ingestive conditioning". "Ingestive conditioning" concerns the modification of a predator's behaviour such that it continues to choose that prey species which it has most recently or most frequently encountered. Such alterations of prey preference may exert considerable effects on the control of local anemone prey populations and their relative abundances. A variant of this experiment was completed during a five week investigation at the Friday Harbor Marine Laboratories, University of Washington, in the summer of 1982. Experiments showed that ingestive conditioning occurs for both U.K. and N.W Pacific A.papillosa. However, the modified experimental design used in Friday Harbor, and in corroborative experiments in St Andrews, also indicate that there is a significant "carry-over" effect from the previous conditioning diet.

In the light of these results it was predicted that A.papillosa might exhibit frequency-dependent (i.e. switching) prey selection. Experiments undertaken to test this hypothesis were unable to demonstrate such behaviour due to persistent preference for one particular prey species.

Field observations of A.papillosa-anemone associations are discussed in relation to the laboratory investigations.

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The nudibranch mollusc Aeolidia papillosa (L.)

AIMS OF THE PROJECT

The objective of this thesis is to define and describe the fundamental characteristics of the relationships between Aeolidia papillosa (L.) and a range of its anemone prey species and to identify determinants of prey-preference behaviour in this nudibranch mollusc.

APPROACH

The first part of this thesis is devoted to an assessment of the nutritional value of a selection of anemone species. This was achieved through laboratory investigation of the anemone tissues themselves, with analysis of the biochemical and calorific composition, and through observations on the effects of mono-specific anemone diets on the growth and reproduction of nudibranchs maintained in the laboratory. In addition, an assessment of diet-related consumption and assimilation patterns have also been undertaken.

Later chapters describe laboratory behavioural experiments which investigated the importance of various factors as determinants of prey-selection by A.papillosa. In particular, the effects of previous dietary experience and prey relative abundances were investigated.

Chapter 1.

AN INTRODUCTION TO THE NUDIBRANCH MOLLUSC AEOLIDIA PAPILLOSA (L.)

The nudibranch mollusc Aeolidia papillosa(L.) is an exclusive predator of sea-anemones and may attain a length of up to 12cm (approx 30g damp weight). This species is geographically widely distributed and can be commonly found on North Atlantic and North West Pacific coasts. It has also been recorded from the Falkland Islands, Chile and Japan, to depths of approximately 800m (Hunnam & Brown,1975). A detailed description of the anatomy of A.papillosa is provided by Alder & Hancock (1855) and Eliot (1910), the latter article being a supplement to Alder and Hancock's series of monographs. In contrast to a number of other nudibranch species there appears to be no taxonomic confusion with respect to A.papillosa, although gross body colouration does vary considerably, particularly in relation to diet. Despite this plasticity of colouration there are certain consistent patterns of pigmentation, such as a white triangle over the head and a dark patch located mid-dorsally over the pericardium. These features permit the reliable recognition of particular individuals.

A.papillosa is a simultaneous hermaphrodite and appears to have an annual life-cycle, although the possibility remains that there may be more than one generation per year. Reproduction is by means of a long-term, dispersive, planktotrophic veliger larva which perhaps requires several weeks or months to complete growth

and development prior to metamorphosis. Spawn masses are usually white and spirally coiled with secondarily twisted egg strings of Type B (Hurst,1967); each egg string consists of a mucopolysaccharide tube containing multi-embryonic egg capsules. Egg diameter in spawn masses laid during this study was approximately 112.6 μ ± 1.86 s.e. Laboratory observation of animals collected from Robin Hood's Bay, North Yorkshire, showed spawning to commence in March and continue through to July. Very small animals were, however, collected from the St Andrews Bay area in all months except April and June-September inclusive, indicating the possibility of a more complex life-cycle.

The veliger larva has a Type 1 larval shell (Thompson,1961) or a Type B veliger shell (Thorson,1946). In common with all opisthobranch veligers the visceral organisation is dextral while the shell is sinistrally coiled. Coiling of the shell differentiates Opisthobranchs from Prosobranchs, because the latter are always dextral. For a detailed description of development and feeding in larval A.papillosa see Williams (1980).

There have been, as yet, no published accounts of successful laboratory culture of A.papillosa veligers through to metamorphosis. Similarly, I was unable to culture A.papillosa veligers for more than three weeks (at 15^{o}C) after their release from the spawn mass. Growth, but no ontogenetic developement, of larvae was observed. Clearly the larva of A.papillosa requires an extended period of planktonic nutrition prior to settlement.

The stimulus for metamorphosis is, however, (in common with many other nudibranch species) likely to be the presence of the live adult diet (see Todd,1981 for review).

Behaviour of A.papillosa, when handling anemone prey, has been described previously by a number of authors (e.g. Russell,1942; Harris,1973; Waters,1973; Edmunds et al.,1976; Harris & Howe,1979). In the initial exploratory phase, A.papillosa approaches an anemone while moving the head from side to side; the oral tentacles are usually extended anteriorly. Detection of prey is apparently achieved in a chemosensory manner and both the oral tentacles and the rhinophores are implicated in this behaviour (e.g Braams & Geelen,1953; Swennen,1961; Wolter,1967; Edmunds et al.,1974). That part of the anemone which is contacted first is largely dependent upon the relative sizes of the predator and prey. If initial contact is made with the anemone tentacle crown those tentacles nearest the nudibranch normally contract and/or retract. Contact with the anemone column, however, may elicit a variable response depending upon the anemone species and the circumstances of the attack. When A.papillosa makes contact with the column of Actinia equina (var. mesembryanthemum), for example, both the column and tentacles nearest the nudibranch contract such that the column bends over toward the predator. This invariably brings the anemone tentacles into contact with the mollusc causing it to withdraw, at least, temporarily (Edmunds et al.,1976). By contrast, contact with the anemone column may cause bulging as a result of exaggerated relaxation of the column wall; this effect

may last for up to 6h for Anthopleura elegantissima (Brandt) (Harris & Howe,1979). This bulging response may raise the tentacles out the of reach of the nudibranch. Harris & Howe (1979) have shown that A.papillosa preferentially attacks the tentacles of A.elegantissima and that the nudibranch may be lifted off the substratum if a tentacle is grasped in the jaws before bulging is initiated. By contrast, Moreteau (1978) has indicated that the nematocyst bearing tentacles of Anemonia viridis(=sulcata) (L.), A.equina and Urticina(=Tealia) felina, are avoided in preference to the column and internal tissues.

Initial contact with the anemone invariably results in the nudibranch retracting the head and thereby the rhinophores and oral tentacles. The cerata are simultaneously "bristled" or temporarily erected. This apparently defensive response is normally more pronounced should contact be made with the tentacles. Following resumption of the attack the anemone tissue is grasped and excised by the jaws; the radula then transports the bolus to the oesophagus. The radula of A.papillosa consists of a single row of broad denticulated teeth.

Biting is normally repeated at 10-20 sec intervals with occasional pauses from feeding activity. These interruptions become longer as feeding progresses and sometimes last several hours. Feeding is continued until the nudibranch is satiated or until the anemone escapes.

A.papillosa, in common with other coelenterate-eating aeolids, has the ability to re-locate functional prey nematocysts in the cnidosacs located at the cerata tips. The literature relating to nematocyst acquisition, storage and utilization has been extensively reviewed by Thompson (1960), Edmunds (1966), Salvini-Plawen (1972), Harris (1973) and Todd (1981). With specific reference to the structure and function of the cnidosac in A.papillosa Kalker and Schmekel (1976) have noted that the arrangement of nematocysts within the cnidophages is species-specific and that the nematocysts lie parallel to one another in A.papillosa. Further reference to A.papillosa is made by Day & Harris (1978), who observed that, for nudibranchs eating Metridium senile (L.), only two of a possible six nematocyst types were relocated in the cnidosacs. Similar patterns of nematocyst selection were also observed for a number of other aeolid nudibranchs.

It is possible that a requirement for nematocysts, as a defense against predators, is a determinant of prey-species selection in A.papillosa. For example, nudibranchs may preferentially select an anemone species in which nematocysts (or the more virulent acontia) are abundant, when stocks in the cnidosacs are depleted. Such possibilities have not, however, been investigated in this study.

Chapter 2.

THE ANEMONE PREY

INTRODUCTION.

For the major part of this study five species of anemone have been used: Sagartia troglodytes (Price), Urticina (=Tealia) felina (L.), Actinia equina (L.) (var.mesembryanthemum), Metridium senile (L.) and Urticina (= Tealia) eques (Gosse). All anemones were collected in the St Andrews Bay area and are common members of the intertidal or sublittoral communities. Three of the species - S.troglodytes, A.equina, and U.felina - are primarily intertidal while M.senile and U.eques are exclusively sublittoral. In view of previous observations that A.papillosa responds differentially to the two colour morphs of Actinia equina it was decided to treat these as separate species throughout. Indeed Haylor et al (1984) have recently suggested that the green morph of A.equina, from the Isle of Man, is a separate species (proposed name: Actinia prasina) and that this might also be true for other Actinia populations. Full descriptions of all these anemones can be found in Manuel (1981).

In addition to studies on British anemone species a five week investigation was undertaken at Friday Harbor Laboratories, University of Washington, U.S.A. with N.W.Pacific A.papillosa and its associated anemone prey. The species used in these experiments were: Anthopleura elegantissima (Brandt), Epiactis prolifera (Verrill), Metridium senile (L.) and Urticina(=Tealia) lofotensis (Danielson). Details of these species can be found in Hand (1955).

Sagartia troglodytes.

S.troglodytes is the smallest anemone in this study and collected specimens rarely exceeded 12mm in basal diameter. Manuel (1981) describes two varieties of S.elegans, var. decorata and var. ornata. The description of var. ornata is most fitting to the anemones used in this study, although, owing to the extreme variability in var. decorata it is possible that some of this variety were used in some instances.

Specimens were collected from among mussel beds located just above M.L.W.S. at Kinkell Braes, East Sands, St Andrews. The most fruitful collecting site was adjacent to a sewage outflow where individuals were abundant and achieved larger sizes. Most of the anemones were attached to shells within mussel clumps or to the rock substratum itself. Exposed anemones could often be found, however, in areas where mussels had been washed away during storms.

Actinia equina (var. mesembryanthemum.)

Most of the A.equina used in experiments were also collected from the Kinkell Braes site. Small A.equina, of comparable size to S.troglodytes, were common among the mussels but larger individuals (up to 30mm basal diameter) were also common. The red morph of A.equina was much more abundant than the green morph and usually extended further up the shore.

Urticina felina.

U.felina, the third intertidal anemone, was collected from beneath rocks, in tide-pools, and in crevices at Kingsbarns Beach, Fife. Collected specimens of this species were usually larger than either S.troglodytes or A.equina (basal diameter up to 40mm) and generally occurred lower down the shore. It was often difficult to obtain sufficiently small individuals for some experiments although smaller specimens could sometimes be found attached to holdfasts of Laminaria digitata (L.).

All three intertidal anemone species are known to be acceptable dietary items for A.papillosa (Waters,1973; Edmunds et al.,1974) and individuals were observed in association with each of these species in the field. During the study period a total of 59 A.papillosa were found at Kinkell Braes in association with S.troglodytes and A.equina. In addition, five A.papillosa were found at Kingsbarns, three of which were associated with

U.felina. Most of the nudibranchs used in this study were, however, collected from Robin Hood's Bay, North Yorkshire. All of the individuals collected from this site were found in association with S.troglodytes.

Metridium senile

M.senile is the only sublittoral anemone that was considered in any detail in this study. Anemones were collected from rocks dredged from the Firth of Forth. Individuals were generally larger than for other species, reaching sizes of up to 100 mm in basal diameter. Small individuals were obtained by maintaining large individuals in aquaria where asexual reproduction, by pedal laceration, sometimes occurred. This behaviour could be encouraged by making small incisions at the base of the attached anemone.

Little information is available on the occurence of M.senile in the St Andrews Bay area although a population has been recorded from the Isle of May. Since M.senile is a widespread anemone which readily forms dense clonal aggregations, localised patches are, perhaps, to be expected.

Urticina eques

U.eques, the other sublittoral species, was only used in preliminary experiments. It was subsequently discarded owing to difficulties in obtaining sufficiently small individuals for experiments.

BIOCHEMICAL AND CALORIFIC ANALYSIS OF ANEMONE PREY SPECIES.

It was expected that this range of (acceptable) prey items would provide inter-specific differences in their 'value' to A.papillosa. Such differences may simply be a consequence of variations in their biochemical composition or they may result from a complex of behavioural and/or ecological characteristics. For example, the particular defensive adaptations of an anemone species may render it especially costly to prey upon in terms of time and energy, thereby reducing its 'value' as a food item. The expectation was that a combination of both biochemical and defensive factors determines the true prey-value to the nudibranch.

In this study prey-values were investigated solely in terms of anemone tissue; that is, from the point at which the nudibranch has overcome the prey and already incurred the costs associated with an attack on a given intact anemone. While remaining aware that ecological and behavioural characteristics of the prey are likely to be important determinants of prey-value for A.papillosa, the investigation of such factors was felt to be outwith the scope of this study. The primary reasons for this were the exceedingly high levels of replication that would be required to adequately account for relative and absolute differences in the size of predator and prey and the various defensive adaptations of anemone species. In addition, considerable problems were encountered in obtaining the desired

quantities of small specimens. A discussion of anemone defensive strategies can be found at the end of this chapter.

The objective of the analyses presented here was to characterise anemone tissues in terms of their gross biochemical composition and calorific content. It was intended that these data would then assist in the interpretation of the results of other experiments in which anemone prey-values are interpreted in terms of growth rate and reproductive output. For example, one might expect A.papillosa to exhibit faster growth rates when feeding on an anemone with an unusually large protein component or with a high calorific value.

MATERIALS AND METHODS.

For the purpose of these analyses it is assumed that there are only minor intra-specific variations in the calorific content or major biochemical components of individuals. In order to minimize such variation tissue samples were obtained by pooling powdered freeze-dried tissue from five individuals. Methods for total protein, lipid and carbohydrate determinations were chosen for their broad sensitivity to components within each class of compounds and for their suitability with the quantities of anemone tissue available.

Total Protein

Protein determinations were performed colourimetrically using the Folin-Lowry method. Full details of this method can be found in Lowry et al.(1951), but a brief outline of the technique will illustrate the salient features. Tissue samples are first dissolved in 0.1M NaOH (0.75mg tissue ml^{-1}) and then incubated with a cupric Copper solution. Folin-Ciocalteau reagent is then added and samples are incubated for a further 30 min before absorbtion of the blue colour is measured at 750nm against a reagent blank. Protein concentrations were determined from a calibration curve using Bovine Serum Albumen as the standard.

Total Carbohydrate

Total carbohydrate was determined according to the method of Dubois et al.(1951). Tissue samples are hydrolysed for 12h at 105°C in 0.5M HCl (2mg tissue ml^{-1}) and the hydrolysates dried in vacuo and resuspended in 10ml of distilled water. Aqueous samples are then mixed with 5% Phenol followed by 96% H_2SO_4. Samples are subsequently allowed to stand for 10min before being incubated at 25°C for a further 20min. Absorption of the yellow/orange colour was measured at 490nm against a reagent blank. Carbohydrate concentrations were determined from a calibration curve using D-glucose as the standard.

Total Lipid

Lipid extraction was performed by a modification of the method of Bligh and Dyer (1959). In this method lipid is extracted from the tissue samples with mixtures of Methanol and Chloroform. The resulting lipid solutions are then rotary evapourated to a small volume and the water was removed using 100% Ethanol as the azeotrope. Lipid concentrations are then determined gravimetrically following evapouration of the solvent on a heating block at $30^{o}C$ under a stream of Nitrogen.

Calorific Analysis

Anemone tissues were prepared for calorimetry by rinsing in isotonic (0.9%) Ammonium Formate (HCO_2NH_4), to remove surface salts, and then freeze-drying (Ammonium Formate sublimes with water ice, when tissues are freeze-dried, leaving no residue). Dry tissue was then finely powdered and mixed before being pressed into pills. Pills were stored at $-20^{o}C$ and re-dried when required.

All calorimetry was performed using a Phillipson Oxygen micro-bomb calorimeter calibrated with benzoic acid standard ($26434.5\ J\ g^{-1}$).

RESULTS.

The results of all analyses are shown in Table 1, and presented graphically in Fig. 1. In all cases protein was the major component ranging from approximately 52% of ash-free dry weight (green A.equina) to approximately 73% (U.felina). Lipid comprised the next largest fraction for S.troglodytes, M.senile, and U.felina although the differences between lipid and carbohydrate values are small (between 2% and 7% respectively). Red A.equina contained approximately equal proportions of both lipid and carbohydrate ($\simeq$10%) while green A.equina was the only species which contained more carbohydrate than lipid.

It can be seen (Table 2) that protein, carbohydrate, and lipid do not account for a constant proportion of the total tissue weight for each anemone species. In the case of S.troglodytes the analysis accounted for only 68% of the total tissue weight. There are a number of possible explanations for these discrepancies. Methods for assessing total protein and carbohydrate exhibit a broad sensitivity to a wide range of compounds within their respective classes. It is quite possible, however, that some proteins or carbohydrates present in specific anemone tissue are not readily detected by these methods. Such effects would underestimate the true composition of the samples. For example, measurement of absorption at 490nm for carbohydrate analysis results in a greater sensitivity for hexose over pentose sugars, thereby underestimating the latter. The presence of

Table 1. Biochemical Composition of Anemone Species (±1 s.e.).

ANEMONE	PROTEIN ($ug\ mg^{-1}$ of O.M)	CARBOHYDRATE ($ug\ mg^{-1}$ of O.M)	LIPID ($ug\ mg^{-1}$ of O.M)
AEQR	0.6498 (±0.0215)	0.1014 (±0.0013)	0.0917 (±0.0103)
AEQG	0.5179 (±0.0091)	0.1597 (±0.0037)	0.1294 (±0.0076)
STRO	0.5288 (±0.0013)	0.0609 (±0.0044)	0.0895 (±0.0075)
MSEN	0.6132 (±0.0039)	0.0746 (±0.0016)	0.1060 (±0.0030)
URFE	0.7283 (±0.0048)	0.0872 (±0.0052)	0.1558 (±0.0074)

For Protein and Carbohydrate analyses n=5.
For Lipid analysis n=3.

Table 2. Percentage of Total Ash-Free Dry Weight Accounted for by Analyses.

AEQR	AEQG	STRO	MSEN	URFE
84.29	80.70	67.29	81.18	97.13

Figure 1.

The biochemical composition of the anemone species calculated as absolute values (a) (ug mg^{-1} of O.M. x 100), proportional values (b), and as calorific composition (c), calculated from the average calorific values for protein, lipid and carbohydrate in invertebrate tissues (from Crisp,1971).

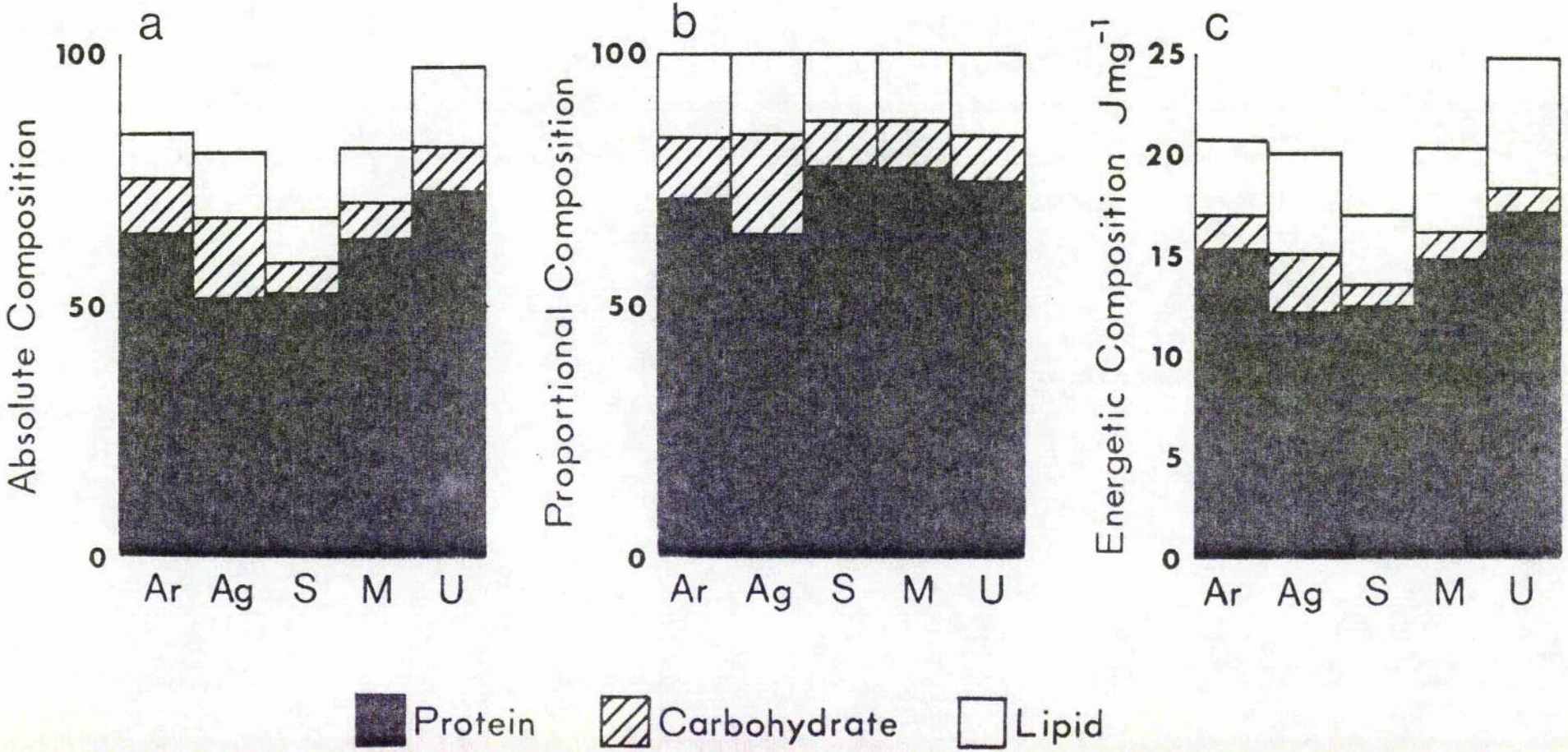
Ar = *Actinia equina* (red morph)

Ag = *Actinia equina* (green morph)

S = *Sagartia troglodytes*

M = *Metridium senile*

U = *Urticina felina*


a
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M
U
Protein
Carbohydrate
Lipid

substances which interfere with the colour reactions may also bias results. In the Folin-Lowry method the presence of, for example, buffers, certain sugars, reducing agents, salts or metals may all interfere with the colour reaction. Finally, the choice of a standard for the calibration curve may affect results since the analysis will tend to be biased toward similar substances in the anemone tissues. The gravimetric method for lipid determinations is generally more robust than the colourimetric methods although not all classes of lipids are extracted with equal ease and it is possible to extract other classes of compound.

Despite the problems associated with methods for analysing such broad classes of compound, on a comparative basis, the results do not indicate any marked differences in the gross biochemical composition of the anemone species (see proportional composition data presented in Fig. 1(b)).

Table 3 shows the calorific values for each species, which ranged from between 1.859×10^4 J g^{-1} ash-free "corrected" dry weight for S.troglodytes to 2.368×10^4 g^{-1} ash-free "corrected" dry weight for red A.equina. The correction is for the endothermic conversion of $CaCO_3$ to $CaCO + CO_2$, which theoretically may account for a 'loss' of 1.799 J mg^{-1} (Paine,1971).

Table 3. Results of Mineral Ash and Calorific Determinations (±1 s.e.).

	Mineral Ash (% of Dry Wgt)	J x 10^4 g^{-1} Dry Weight	J x 10^4 g^{-1} Ash-Free Dry Weight	J x 10^4 g^{-1} Ash-Free Dry Wgt"corrected"
AEQR	10.506 ± 0.158	2.099 ± 0.020	2.347 ± 0.022	2.368 ± 0.022
AEQG	10.694 ± 0.038	2.073 ± 0.012	2.321 ± 0.013	2.343 ± 0.013
STRO	8.852 ± 0.143	1.680 ± 0.016	1.842 ± 0.018	1.859 ± 0.018
MSEN	13.326 ± 0.124	2.014 ± 0.008	2.324 ± 0.009	2.351 ± 0.009
URFE	10.624 ± 0.059	1.889 ± 0.015	2.112 ± 0.017	2.133 ± 0.017

For all cases n=7 except for STRO where n=5.

In addition to the absolute and relative composition of the anemone species, Fig. 1(c) also shows the calorific contribution of the three biochemical components. These data were calculated from average calorific values for proteins, lipids and carbohydrates for a range of invertebrate tissues (Crisp,1971). The figure demonstrates the high calorific contribution of protein, and especially lipid, in contrast to carbohydrate.

DISCUSSION.

On a proportional basis the biochemical analyses do not indicate any marked differences between the anemone prey species. The histograms of absolute biochemical composition do, however, show apparent differences between, for example, U.felina and S.troglodytes. Nevertheless, it should be emphasised that a greater component of dry tissue weight was accounted for in U.felina. Consideration of the calorific conversions (Fig. 1(c)) from the biochemical analysis compared with the micro-calorimetric determinations (Table 3) shows values differing by only 0.25 - 0.37 x 10^4 J g^{-1} for each species. It is concluded, therefore, that these analyses corroborate one another and neither provides supportive evidence for marked differences in biochemical prey values of any species to A.papillosa. Differences in tissue values (i.e. after prey defenses have been overcome) may, however, result from species-specific differences in assimilation efficiency and/or

rate of consumption by the nudibranch. (These possibilities are considered in Chapter 4). In addition, prey values may differ as a result of qualitative and/or quantitative differences in specific nutritional components (e.g. essential amino acids, trace elements, vitamins, etc.). Such factors are indicated as being important in the case of the wolf spider Pardosa ramulosa (McCook) which has been shown to prey upon three species in proportions which optimise the proportions of essential amino acids in the diet (Greenstone,1979). The present study does not extend to the inclusion of such specific components.

DEFENSIVE STRATEGIES OF ANEMONE SPECIES.

A wide variety of strategies, which act to deter predatory attacks by A.papillosa can be observed among the anemone species. These strategies can be considered as divisible into two categories: notably, i) defensive features associated with the habitat in which the anemone occurs, and ii) species-specific defense and escape resonses which are elicited when encountered by a nudibranch. The former class of strategy, and its effectiveness in deterring A.papillosa predation, has been considered for a range of anemone species by Waters (1973) and Edmunds et al. (1976). Both studies demonstrated species-specific defensive and escape behaviour in response to A.papillosa attack. A summary of the data of Edmunds et al. (1976) is presented in Table 4.

The defensive strategy of each anemone species can be considered as a 'cost' to A.papillosa, since time and energy must be spent in overcoming the chosen prey. This 'cost' can be considered as being largely analagous to the concept of 'handling time' that has been advanced in many optimal foraging models (for reviews see, for example, Pyke et al.,1977; and Hughes,1980). It is reasonable to suggest that variations in the defensive strategy of particular anemone species, and thus in 'cost' through handling time for A.papillosa, will be an important determinant of prey choice in the nudibranch. As discussed previously, however, an interpretable quantitative determination

Table 4. Responses of anemones to attack by A.papillosa (from Edmunds et al.,1976).

	Tentacle retraction	Tentacle waving & stinging	Column shortening	Column inflation	Pedal disc withdrawal	Locomotion	Detachment	Ejection of acontia
A.equina (var. mesembryanthemum)	••	–	••	••	••	••	••	
A.equina (var. fragacea)	••	–	••	••	••	••	••	–
Anemonia sulcata	–	••	–	–	••	••	–	–
Anthopleura elegantissima	••	–	••	••	••	••	••	–
Anthopleura balli	••	–	••	••	?	?	?	–
Urticina felina	••	–	••	•	•	•	•	–
Sagartia elegans	••	–	••	•	–	–	•	••
Cereus pedunculatus	••	–	••	–	–	–	?	••
Aiptasia couchi	••	–	••	?	?	?	•	••
Corynactis viridus	••	–	••	?	?	?	?	-
Actinothoe sphyrodeta	••	–	••	•	?	?	•	••
Metridium senile	••	–	••	•	•	•	•	••

of such 'costs' - as exemplified by handling times and attack success - would be most difficult to achieve. Little can presently be added to the reports of Waters (1973), and Edmunds et al. (1976), although one observation does indicate that there may be a markedly greater risk for juvenile A.papillosa attacking M.senile. In June 1981 one nudibranch (≏2g damp wt) became tangled in the nematocyst-bearing mesenteric filaments from a piece of M.senile that was provided as food. The nudibranch was unable to disentangle itself from the acontia and died three days later. By contrast, another nudibranch (≏10g damp wt) which became similarly entangled, succesfully sloughed off the acontia and mucus within 24h. It is to be expected that acontian anemones, which appear to possess more virulent nematocysts than their actinian counterparts, will pose more of a threat to juvenile A.papillosa, which choose to prey upon them.

Personal observations of the defensive strategies of the anemones in this study are in general agreement with those of Edmunds et al. (1976), particularly with regard to the difficulty A.papillosa encounters in attacking inflated and detached A.equina. Responses differ, however, between species. For example, A.equina often inflates and detaches in response to an attack, while U.felina will inflate but rarely detach.

The relative size of both predator and prey may also affect the nature of prey responses and their effectiveness as a deterent to A.papillosa. While small nudibranchs, in themselves, are unlikely to pose a threat to large anemones the damage

incurred in such attacks may be sufficient to attract other aeolids, thereby increasing the probability that the anemone will be killed.

The effect of anemone habitat on nudibranch predation is especially difficult to assess. It is to be expected that specific anemone habitat choice is primarily the result of such factors as the environmental tolerance and feeding characteristics of their species, rather than effectiveness in deterring A.papillosa predation. This does not, however, preclude the possibility that habitat preferences have a bearing on the defense of anemone species. One would expect that defense and escape behaviour would evolve to incorporate the constraints or advantages of habitat preference to form an effective defense strategy. It may be more difficult, for example, for A.papillosa to overcome S.troglodytes, which is often buried among mussels and sediment, than to overcome A.equina which may be sympatric but never buries. Such factors may be offset, however, by the fact that A.equina, unlike S.troglodytes, readily inflates and detaches to escape predation. Detachment may, however, incur considerable risk for an anemone; in the high energy environment of the intertidal re-attachment in a suitable location may not be possible. Despite this, it is likely that the risks associated with detachment are outweighed by a high probability of death in a confrontation with A.papillosa.

U.felina provides a further example of how habitat may provide at least partial protection from predation. This species is normally found in rock pools and crevices in the lower intertidal and is often prevalent in areas where coarse shell gravel accumulates. Fragments of shell gravel are often adherent, on epidermal cinclides, to most of the surface of the anemone column. This undoubtedly presents a considerable obstacle for A.papillosa, and it is possible that for some contracted anemones the shell layer may confer complete protection.

One further defensive adaptation has been demonstrated for the North American anemone Anthopleura elegantissima. This species utilises an 'alarm pheromone' which is released from nudibranchs which have recently preyed upon this anemone. Alarm responses may be elicited in other conspecific anemones approached by the nudibranch for up to five days after a meal (Harris,1976; Howe & Harris,1978; Harris & Howe,1979).

Such defensive adaptations displayed by the anemone species are certain to be important determinants of predatory behaviour in A.papillosa. The evolution of such adaptations has resulted in a complex range of interactions which will require extensive study before their effects on the fitness of A.papillosa can be determined. Some progress has been made in this regard for Californian A.papillosa. In investigating the prey associations of the nudibranch Harris & Howe (1979) undertook a variety of

experimental approaches in order to explain the preponderance of field associations between A.papillosa and the cloning sublittoral anemone M.senile. (A.elegantissima had been indicated as being the preferred prey in several studies (Harris,1976; Howe & Harris,1978; Harris & Howe,1979). Their conclusions can be summarised by stating that A.papillosa appears to be evolving to specialize on M.senile since the behavioural, chemical (nematocyst and 'alarm pheromone') and distributional defences of the preferred item, A.elegantissima, are sufficient to exert a strong negative selection pressure on associated A.papillosa: i.e. larvae settling on A.elegantissima seldom attain maturity and reproduce, and as such are of low or negligible fitness in contrast to M.senile associates. Further studies of this kind are required before reliable conclusions can be drawn about this aspect of predator-prey interactions for European A.papillosa.

Chapter 3.

THE EFFECTS OF ANEMONE DIET ON GROWTH AND REPRODUCTION IN AEOLIDIA PAPILLOSA (L.)

INTRODUCTION.

In the absence of data on the numbers of individuals which survive to reproduction in the following generation, perhaps the best measure of inclusive fitness is in terms of the growth and reproductive output of an individual over its entire life-cycle. Intuitively, one would expect food quality to affect growth and also reproductive output: the latter generally increases with body size (Todd & Havenhand,1983). The objective of the observations described in this chapter is to determine the effects of mono-specific anemone diets on these measures of fitness, thereby providing a test for differences in prey-value at its most fundamental level.

A relationship between food quality and growth is, perhaps, unsurprising but has nevertheless been demonstrated in molluscs by Leighton & Boolootian (1963), for a prosobranch, and by both Chia & Skeel (1973) and Bloom (1974) for opisthobranchs; all of these studies showed an increased growth rate for molluscs which were fed to excess on their preferred food. A positive relationship between body size and reproductive output has been clearly demonstrated for a number of nudibranch species (Todd &

Havenhand,1983) and, although egg production is, perhaps, a more direct measure of inclusive fitness than growth, the latter may be an important fitness parameter. This may be the case if, for example, the expectation of survival under unfavourable field conditions is positively correlated with body size. Growth and reproduction must, therefore, be considered together if a realistic estimate of inclusive fitness is to be obtained.

Laboratory studies of growth in opisthobranchs have been reported by a number of authors (Paine,1965; Carefoot,1967; Holleman,1972; Chia & Skeel,1973; Bloom,1974; Todd,1979b) and a more general account of growth in the Mollusca is provided by Wilbur & Owen (1964). Of these, the studies of Paine (1965) and Carefoot (1967) specifically relate growth to the nutritional characteristics of the food. Field estimates of opisthobranch growth rates include those of Miller (1962), Paine (1965), Potts (1970), and Todd (1979b).

The interpretation of field measurements of growth in a realistic ecological context is undoubtedly easier than for data collected in the laboratory. In the present context, however, the interest in growth rates lies primarily in the detection of differential growth responses in relation to food type; laboratory estimates of growth are, therefore, more appropriate.

Reproductive effort in opisthobranchs is a subject area of growing interest, particularly in relation to life-history strategies. Thus far, published data are available for only four species: Todd & Havenhand (1983) provide a valuable summary of these data and discuss the definition and measurement of reproductive effort along with its interpretation, particularly in relation to gastropod life-history strategies. In the present context, both reproductive output (total egg production) and reproductive effort (energy allocated to reproduction/energy allocated to somatic growth) are considered in relation to anemone diet.

In this analysis the effects of anemone defensive responses on prey-value to A.papillosa have not been considered: i.e. prey-values are assessed from the point at which anemone defences are assumed to have been overcome. The observations represent, therefore, an analysis of prey-values in terms of their physical and biochemical characteristics. Such an analysis is an essential prerequisite to quantitative investigations of anemone defensive responses as determinants of prey-value, and thus, prey-choice in A.papillosa.

MATERIALS AND METHODS.

Both growth and reproductive output of individuals were monitored throughout most of the life-cycle, from November 1982 to July

1983. Data are, however, lacking for the first few weeks of benthic life prior to collection from the field.

A total of 39 juvenile nudibranchs were collected from Robin Hood's Bay, North Yorkshire in early October 1982 and were divided between six diet treatments. A further nine juveniles were collected from East Sands, St Andrews, one month later and were included in the analysis to provide a total of eight nudibranchs per treatment. The weights of nudibranchs on collection ranged from 0.006g to 0.330g (mean = 0.0967±0.0119 s.e.) and allocation of individuals to treatments was balanced so that each group contained a similar range of initial mollusc weights. No differences in performance were detected between nudibranchs collected from the two field locations.

Animals were maintained throughout the experiment in 2.5l (0.5 gallon) containers with a water depth of 2.5 cm. All containers were located in a "cascade" system through which fresh seawater continually flowed. The containers were divided, initially, into two equal parts by a mesh screen separator and two individuals were maintained in each half. Very small nudibranchs, however, were initially kept in individual plastic mesh cages within the system because the mesh size of the partitions was too large to prevent them escaping. Individual nudibranchs were identifiable from detailed notes of body markings.

With the onset of spawning it became necessary to further sub-divide each container so that all four individuals were held in isolation: this allowed individual spawn production to be monitored. The following anemone species were used:

Actinia equina (Red Morph)

Actinia equina (Green Morph)

Sagartia troglodytes

Metridium senile

Urticina felina

Five of the treatment groups were fed on cut pieces of a single anemone species throughout the experiment, while the final group was fed on a sequential mixture of all five species. To ensure that all species were eaten in the mixed diet group anemones were provided in rotation for periods of two weeks each.

All animals were damp-weighed every fortnight throughout the experiment. Excess water was removed by rolling the animal on a plastic gauze overlying dry filter paper. Repeat damp weighing of a range of nudibranch sizes showed a variation about the mean of approximately 1%. Throughout the year A.papillosa spanning a range of sizes were collected from the St Andrews Bay area. These individuals were damp-weighed and then rinsed in 0.9% Ammonium Formate to remove surface salts (this solution is isotonic with seawater and the Ammonium formate sublimes totally on freeze-drying). Freeze-dried individuals were then re-weighed to provide a damp/dry weight conversion factor.

After the first nudibranch had spawned, individuals were separated as described above. On two or three occasions each week nudibranchs within treatments were paired for six to eight hours to permit copulation. All animals were examined daily and spawn mass production was recorded. Spawn masses were generally laid on the sides of the container and were easily excised using a sharp scalpel and fine forceps. All spawn masses were blotted and damp weighed, prior to being freeze-dried to provide damp/dry weight conversion data and material for calorific analysis.

For investigation of reproductive effort, inorganic ash content and calorific values were determined for pre-reproductive A.papillosa and intact spawn. The methods used were the same as those described for the analysis of anemone species in Chapter 2.

RESULTS.

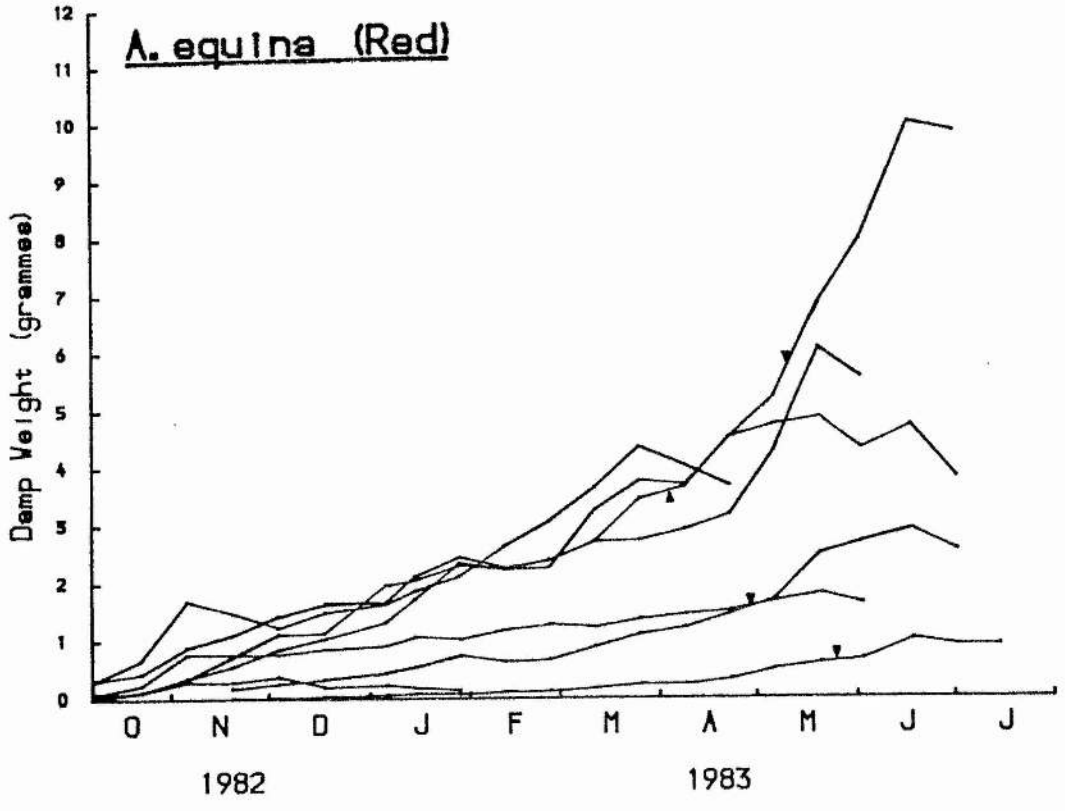
Figs. 2 (a),(b) and (c) present the growth data for individual nudibranchs in each of the diet treatments and, for the 21 nudibranchs which reached reproductive maturity and spawned, the date on which the first spawn mass was laid (▼). Perhaps the most striking feature of these data is the variability in the growth of individuals even within a given diet treatment. For example, after a period of 183 days from the start of the experiment there was a difference in damp weight of 9.413g between the largest and smallest individuals maintained on

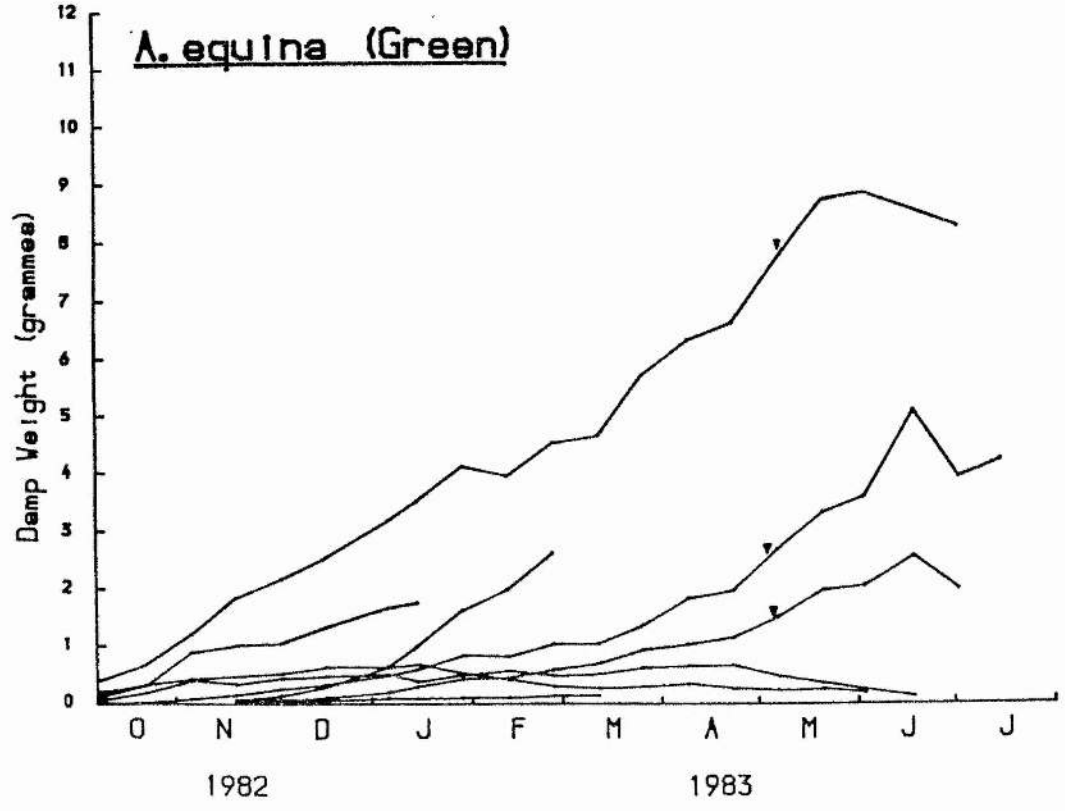
Figure 2(a), (b) and (c).

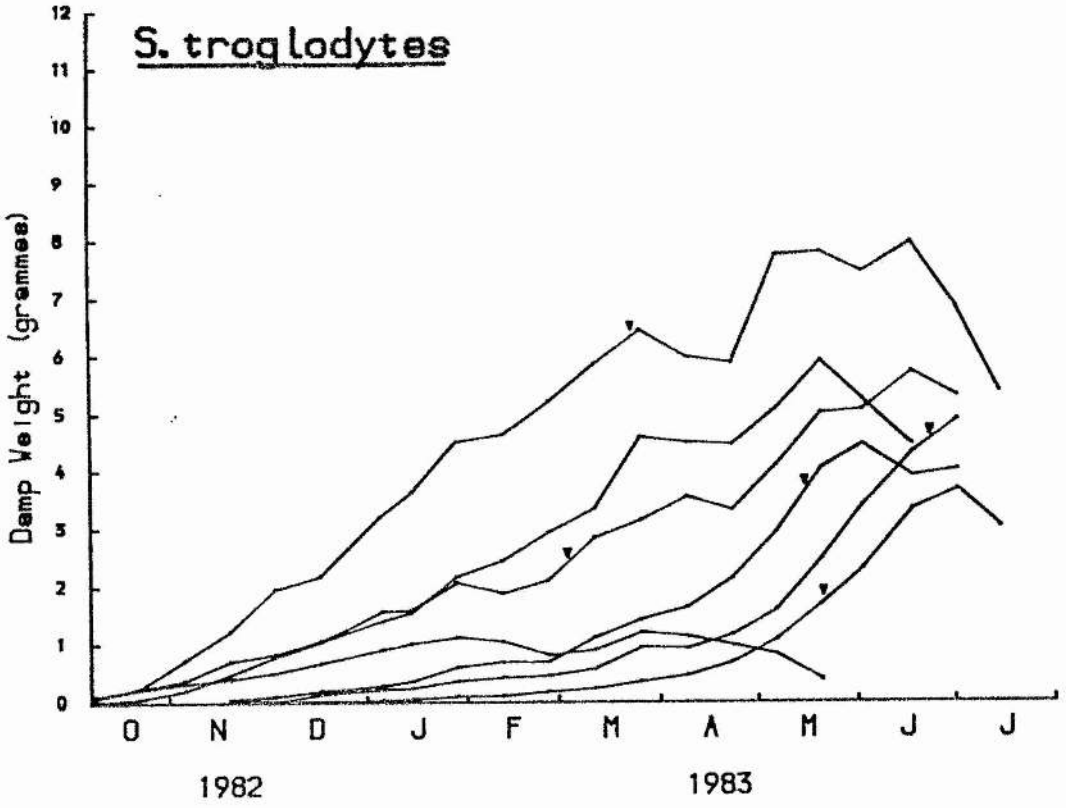
Growth data for individual nudibranchs in each diet treatment.

(▼) Denotes date of first spawning.

2a


A. equina (Red)
Damp Weight (grammes)
0
1
2
3
4
5
6
7
8
9
10
11
12
O
N
D
J
F
M
A
M
J
J
1982
1983


A. equina (Green)
Damp Weight (grammes)
0
1
2
3
4
5
6
7
8
9
10
11
12
O
N
D
J
F
M
A
M
J
J
1982
1983


S. troglodytes
Damp Weight (grammes)
0
1
2
3
4
5
6
7
8
9
10
11
12
O
N
D
J
F
M
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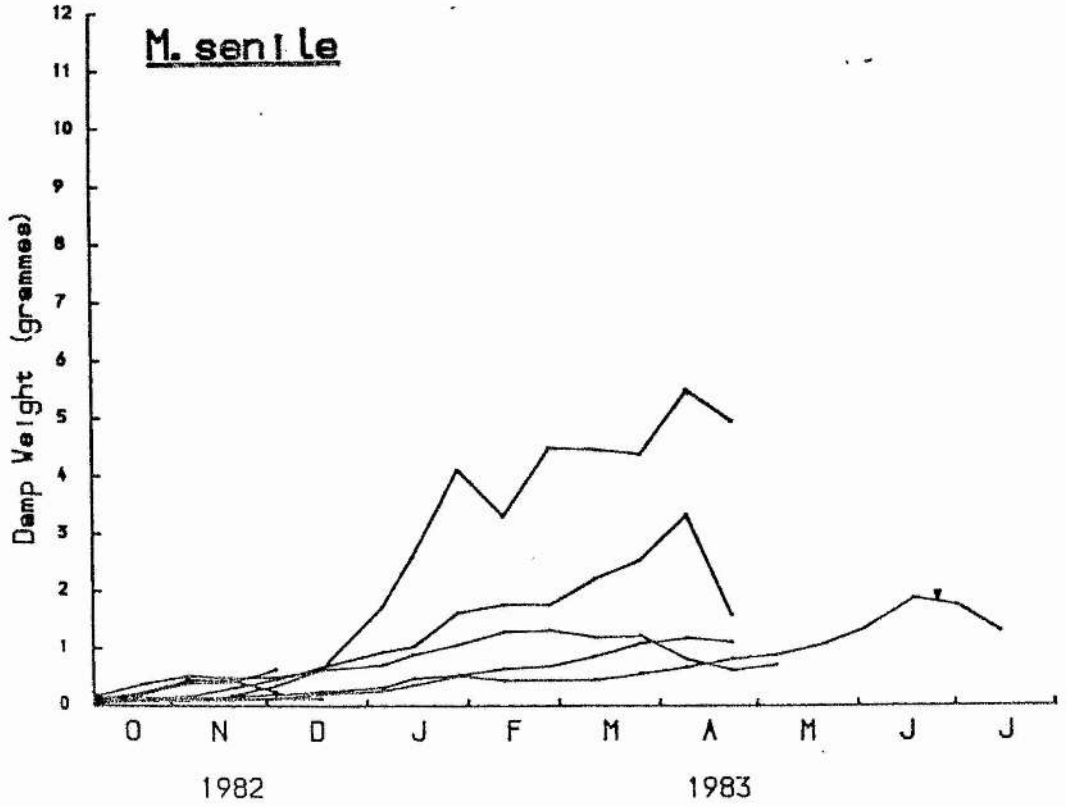
M
J
J
1982
1983


M. senile
Damp Weight (grammes)
0
1
2
3
4
5
6
7
8
9
10
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12
O
N
D
J
F
M
A
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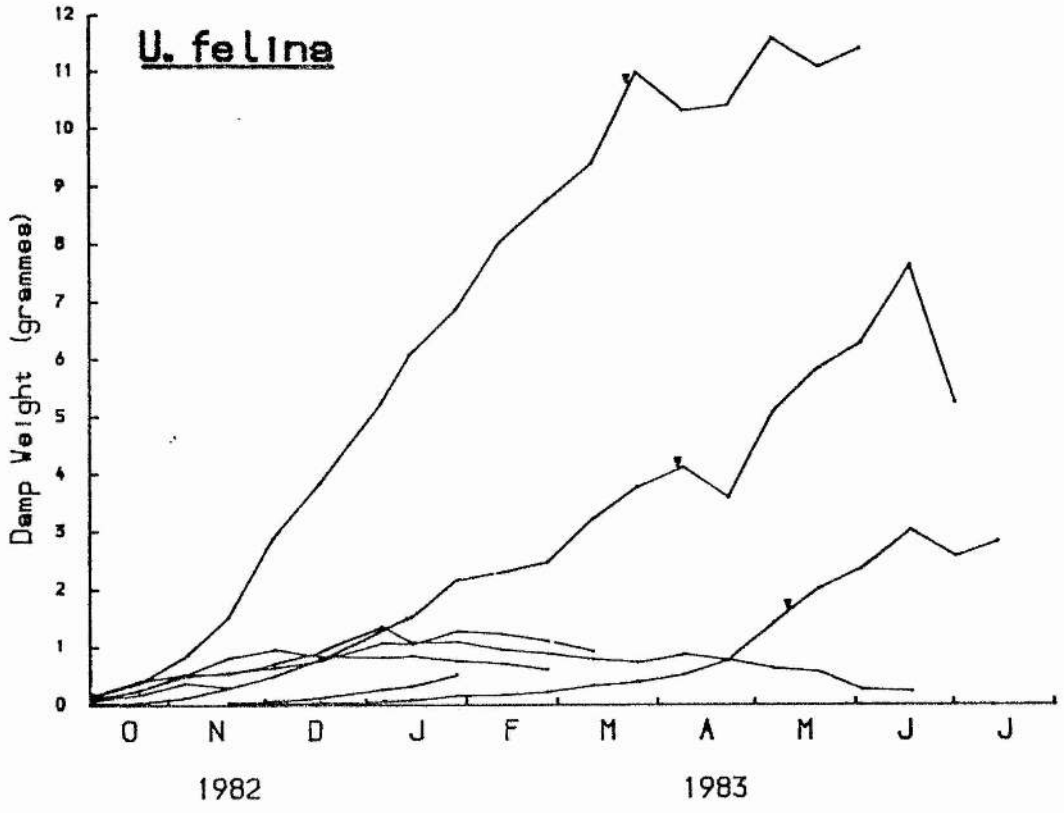
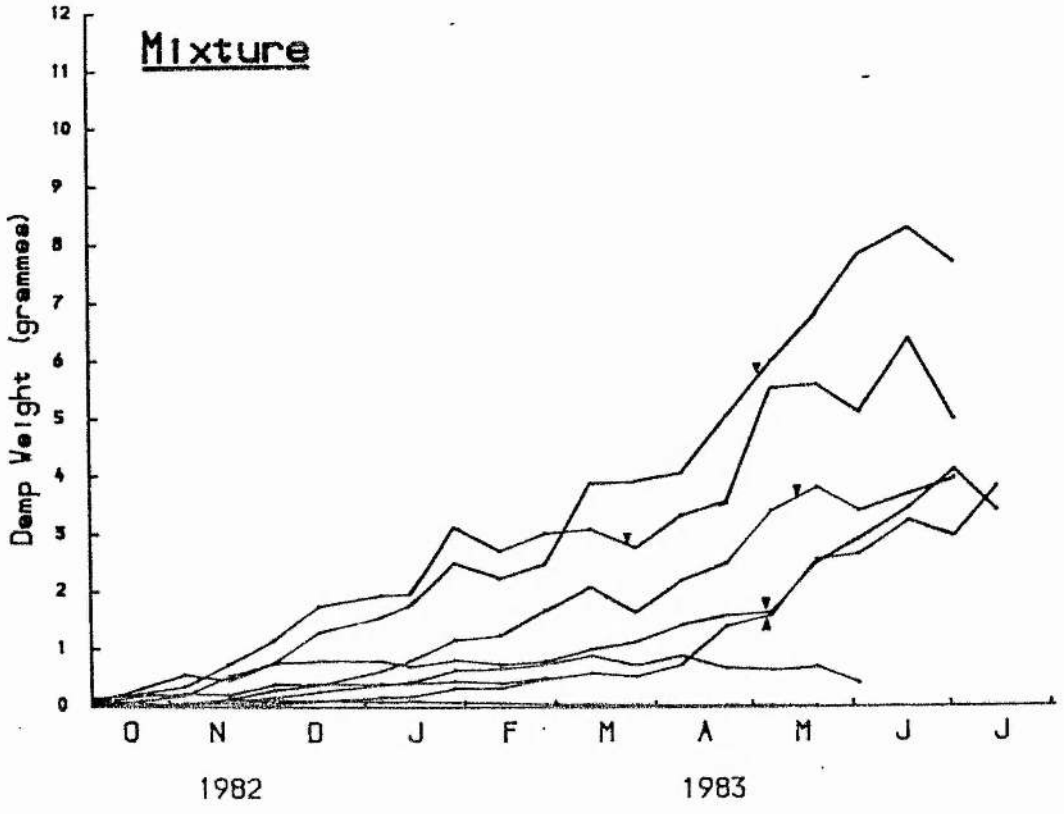
J
J
1982
1983


U. felina
Damp Weight (grammes)
0
1
2
3
4
5
6
7
8
9
10
11
12
O
N
D
J
F
M
A
M
J
J
1982
1983


Mixture
Damp Weight (grammes)
0
1
2
3
4
5
6
7
8
9
10
11
12
O
N
D
J
F
M
A
M
J
J
1982
1983

U.felina. The respective damp weights of these two nudibranchs at the beginning of the experiment were 0.154g and 0.181g. Examination of the whole data set show other, equally striking, differences in growth rate.

The large variation in growth of individuals within each diet set preclude any suggestion of dietary effects. The apparent absence of such trends may be attributable to small sample sizes.

Although growth rates appear to be unaffected by the anemone diets the results do suggest that survivorship is reduced for A.papillosa maintained on M.senile alone. Only one M.senile-fed animal survived beyond early May 1983, in contrast to all other treatments in which four or more nudibranchs survived until at least mid-June 1983. The treatment group sizes, however, are too small to draw any firm conclusions regarding survivorship. It should be noted that this feature of these data may be the consequence of a greater tendency for cut pieces of M.senile to decay in the aquarium. Although the food of all animals was regularly monitored in an attempt to ensure that it was in a satisfactory condition, decay of M.senile over the one or two day period between inspections may have been sufficient to depress the gross survivorship of nudibranchs feeding on that species. There was rarely, however, any visible evidence of decay on the M.senile pieces that were removed.

All but one of the nudibranchs which spawned continued to grow after the onset of reproduction. In four cases the maximum post-spawning body size was attained just prior to death. For the remaining nudibranchs a fall in weight was generally recorded approximately two weeks before death.

In order to permit analysis of patterns of energy partitioning between reproduction and somatic growth all damp weights for spawn masses and adults were converted to dry weights using the conversions shown below: these were obtained from least squares regression analyses of damp weight(y) on dry weight(x).

Soma Dry Weight(x) = (Damp Weight(y) / 8.9030) + 0.1898)
$r=0.9951^{***}$, $r^2=0.9902$, $n=22$.

Spawn Dry Weight(x) = (Damp Weight(y) / 10.04809) - 0.0118
$r=0.9889^{***}$, $r^2=0.9779$, $n=25$.

Dry weights were then converted into calorific (joule) equivalents; the units referred to hereafter.

Table 5 shows the mineral ash and calorific values used in the conversions for both somatic tissue and spawn. One error implied in these analyses arises with the use of the calorific conversion factor for somatic tissue on reproductively mature nudibranchs. Some of the measured weight of these animals may be contributed by the gonad and thereby an over-estimation of total

Table 5. Results of mineral ash and energetic determinations for somatic tissue and spawn of A.papillosa (± 1 s.e.)

	Mineral Ash %	J mg^{-1} Dry Wt	J mg^{-1} Ash Free Dry Wt	J mg^{-1} Ash Free Dry Wt corrected
Soma	16.938 ± 0.0916	17.305±0.2920	20.833±0.351	21.201±0.351
Spawn	16.455 ± 0.6516*	19.245±0.141	23.035±0.169	23.050±0.169

n = 5 in all cases except *, where n = 6.

somatic production may have been obtained. Without information on the internal partitioning of reproductive and somatic tissues at the time of weighing, however, this error is unavoidable. From a comparative viewpoint the results remain valid.

Table 6 summarizes the body size, spawning and reproductive effort data for the 21 nudibranchs which attained reproductive maturity and spawned. The first spawn mass was laid on 2nd March,1983 although the majority of nudibranchs did not commence spawning until May and continued until their death in early/mid July. A total of 171 spawn masses were laid by the 21 animals of which only 8 were infertile. The infertile spawn masses were included in the analyses since they still represent energy allocated to reproduction and may have been the result of insufficient insemination or some other laboratory effect. In this context it is relevant that A.papillosa denied access to a mate fail to spawn before death in contrast to, for example, Onchidoris bilamellata (L.) which exerts a significantly greater 'reproductive effort' when maintained in isolation (Todd,1979b). The smallest animal to spawn weighed 1.647g: four nudibranchs which failed to spawn exceeded this size only after the beginning of May, when the remainder had commenced reproduction. Infertile spawn masses appeared sporadically and could not be related to diet treatment or particular individuals; their inclusion has little effect on the reproductive patterns which emerge.

Table 6. Summary of Body Size Spawning and Reproductive effort Data

	Max Damp Wt(g) with Date	No of Spawnings	Date of First Spawning	Date of Last Spawning	Duration of Spawning (d)	Max Post-Spawn Body J x 10^4	Total Spawn J x 10^4	R.E % (Turnover ratio)
	8.832 (02.06.83)	6	07.05.83	07.07.83	61	2.0451	3.0481	149.0
AEQR	2.559 (17.06.83)	10	04.05.83	13.07.83	70	0.8258	0.8751	105.9
	5.089 (17.06.83)	12	03.05.83	21.07.83	79	1.3176	2.6629	202.1
	4.867 (20.05.83)	9	02.04.83	04.07.83	93	1.2744	1.7571	137.9
AEQG	9.988 (17.06.83)	6	09.05.83	12.07.83	64	2.2698	2.5890	114.1
	1.026 (17.06.83)	7	26.05.83	21.07.83	56	0.5278	0.2708	51.3
	2.921 (17.06.83)	8	30.04.83	10.07.83	71	0.8962	0.8534	95.2
	4.877 (01.07.83)	2	25.06.83	06.07.83	42	1.2764	0.4635	36.3
	7.950 (17.06.83)	11	24.03.83	15.07.83	113	1.8737	4.5702	243.9
STRO	5.691 (17.06.83)	9	02.03.83	05.07.83	125	1.4346	2.4102	168.0
	4.446 (02.06.83)	10	16.05.83	10.07.83	55	1.1926	2.4670	206.8
	3.666 (01.07.83)	11	23.05.83	13.07.83	51	1.0410	1.6490	158.4
MSEN	1.747 (01.07.83)	4	28.06.83	14.07.83	16	0.6680	0.4766	71.3
	11.549 (06.05.83)	5	22.03.83	22.05.83	61	2.5732	2.2269	86.5
URFE	7.621 (17.06.83)	6	05.04.83	05.07.83	91	1.8097	2.1054	116.3
	3.025 (17.06.83)	12	11.05.83	16.07.83	66	0.9164	1.6552	180.6
	6.355 (17.06.83)	8	21.03.83	08.07.83	109	1.5597	2.5732	164.9
	8.269 (17.06.83)	9	02.05.83	11.07.83	70	1.9537	3.6541	188.8
MIXTURE	4.080 (01.07.83)	12	05.05.83	13.07.83	69	1.1214	1.8948	168.9
	3.945 (01.07.83)	7	12.05.83	10.07.83	59	1.0952	1.3626	124.4
	3.795 (14.07.83)	6	05.05.83	04.07.83	60	1.0660	1.0821	101.5

Spawn-mass size was comparatively constant over the whole of the spawning period, although the final spawn mass was often smaller than the others. Fig. 3 shows the relationship between maximum post-spawning body size and mean spawn-mass size. Maximum post-spawning body size is considered to be the most appropriate static measure of somatic production in determining turnover ratios. A highly significant linear relationship between the mean spawn mass size(y) and the maximum post-spawning body size(x) is detectable:

$$y = 2.359x - 0.7356,\ r=0.9542^{***},\ r^2=0.9104,\ n=21.$$

Inspection of the pooled data in Fig. 3 suggests that there is a maximum mean spawn mass size resulting in a plateau for individuals greater than 1.9×10^4 J (9.0g damp wt). The plateau in mean spawn mass size appears to be approximately 4×10^3 Joules.

Fig. 4 summarizes the mean temporal patterns of spawn production for 14 nudibranchs for the first 60 days following the commencement of spawning (none of these animals survived beyond 70 days). The pattern of increasing rate of spawn production as the spawning period progresses is a consistent feature for all but two of the 21 nudibranchs which were observed spawning. This pattern differs markedly from that demonstrated by other nudibranch species. Todd (1979a) has observed two contrasting patterns of spawn production for the dorid nudibranchs Onchidoris

Figure 3.

The relationship between body size and mean spawn mass size (±1 s.e.).

The numbers above or below the error bars indicate the number of samples.

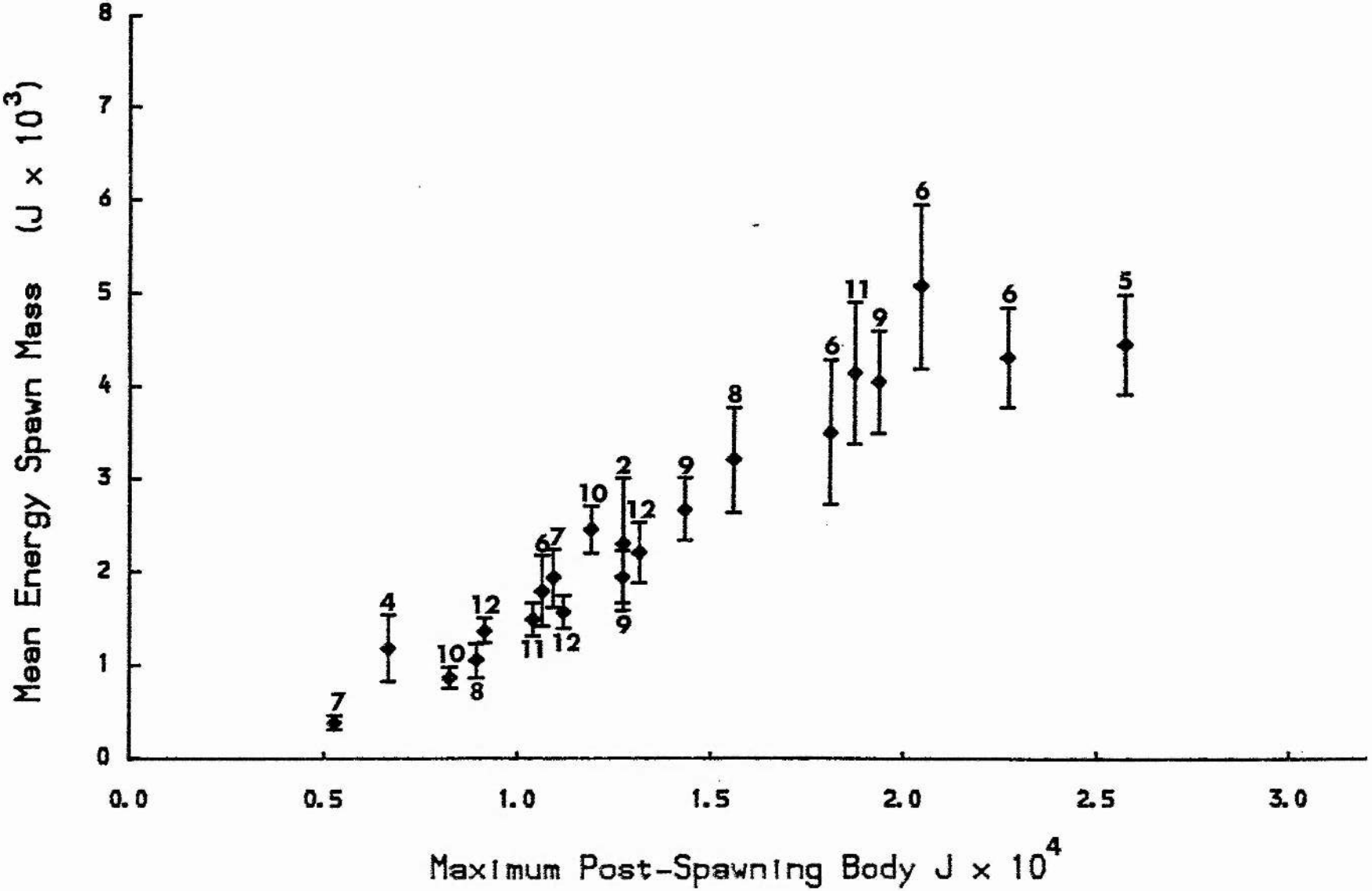
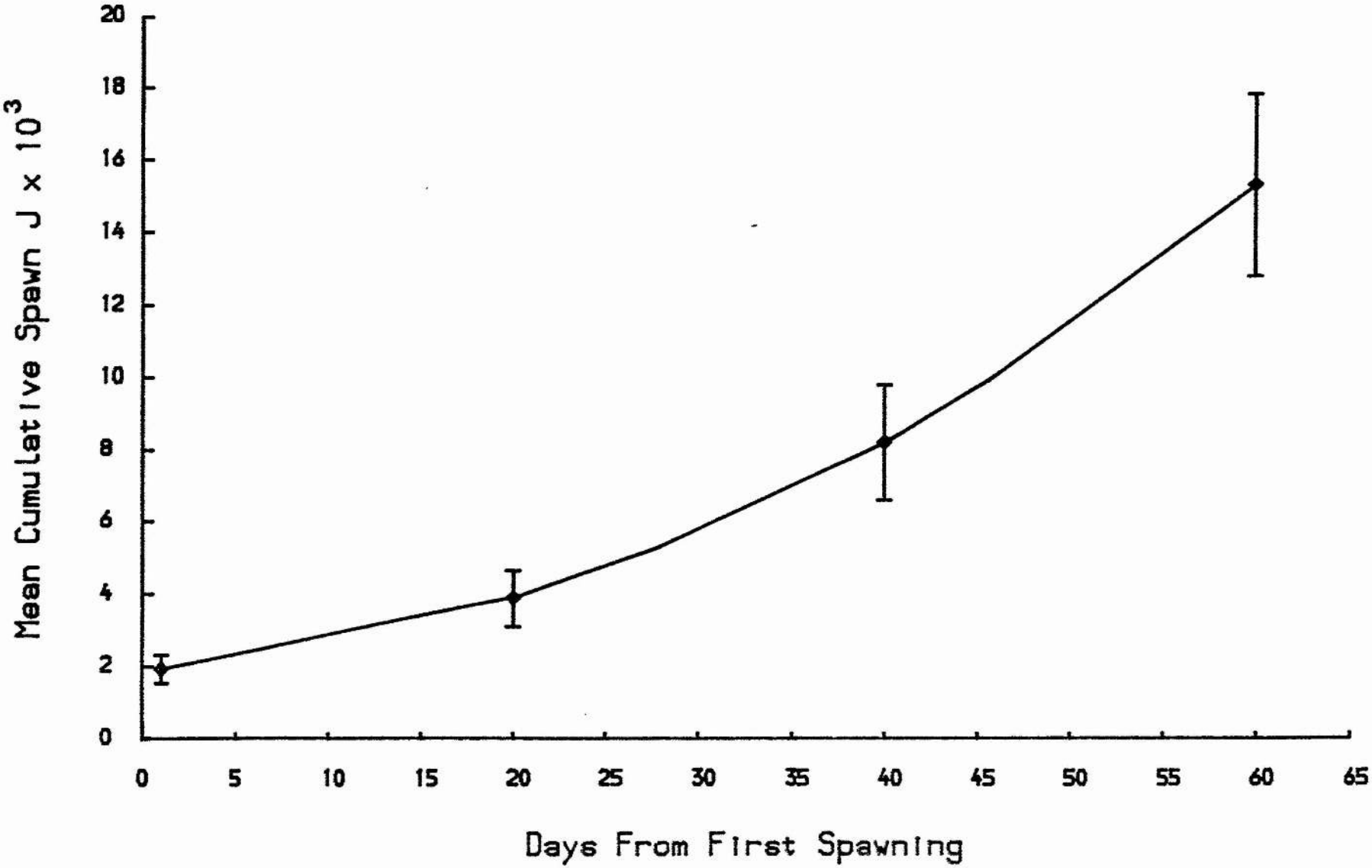

Mean Energy Spawn Mass (J x 10^3)
Maximum Post-Spawning Body J x 10^4
0
1
2
3
4
5
6
7
8
0.0
0.5
1.0
1.5
2.0
2.5
3.0

Figure 4.

Mean cumulative spawn production (±1 s.e.) for 14 nudibranchs over a period of 60 days. (No individual survived beyond 70 days).


Mean Cumulative Spawn J x 10^3
20
18
16
14
12
10
8
6
4
2
0
0
5
10
15
20
25
30
35
40
45
50
55
60
65
Days From First Spawning

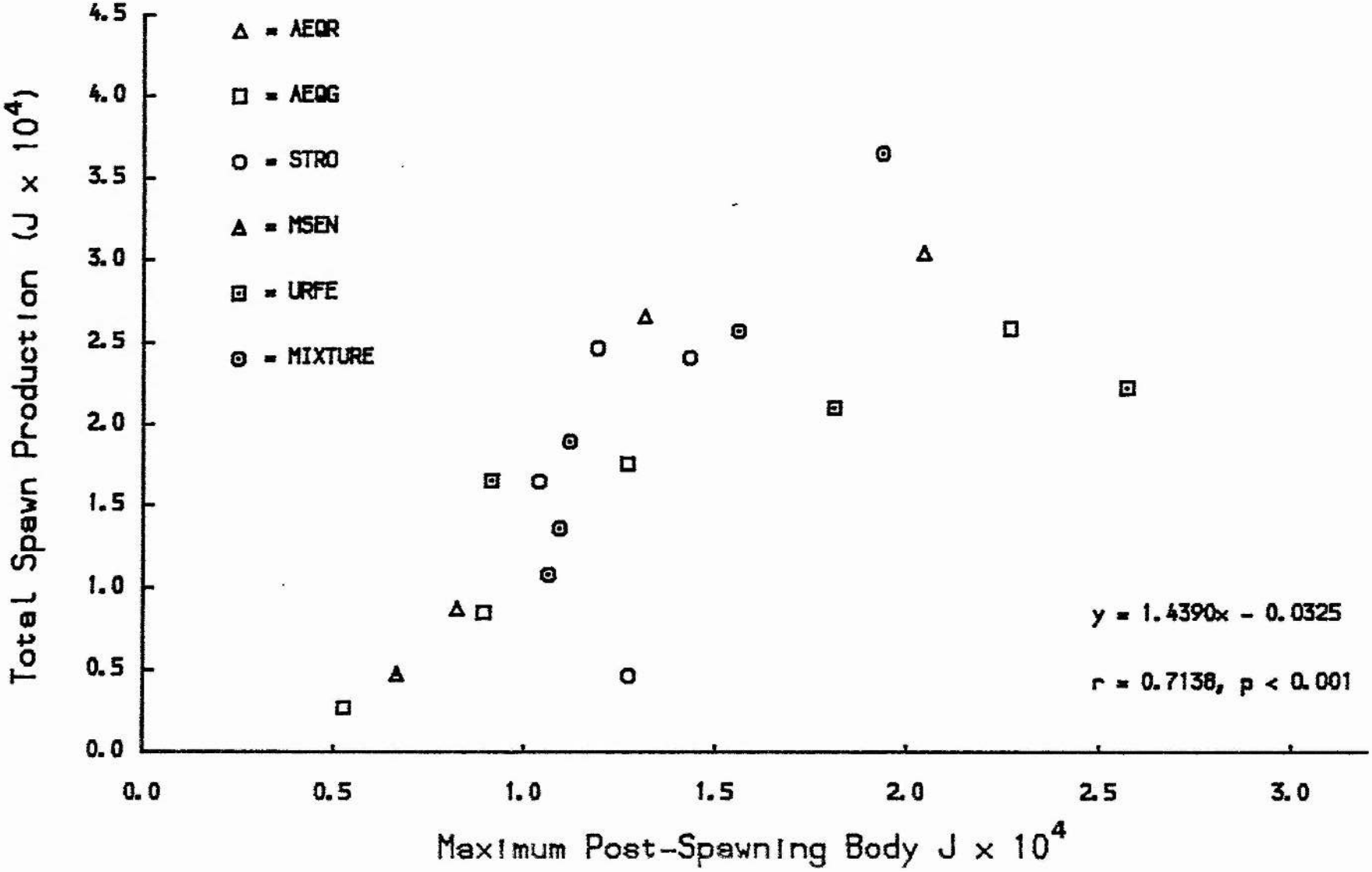
muricata (Müller) and Adalaria proxima (Alder & Hancock). Cumulative spawn production for pairs of O.muricata can be described by a power curve equation in which the rate of spawn production decreases over time (the converse of the pattern observed for A.papillosa). In contrast, the cumulative spawn output of A.proxima shows a sigmoid relationship with time. A similar pattern to that demonstrated for O.muricata has also been observed in O.bilamellata (Todd,1979b).

The absolute rate of spawn production in A.papillosa is very variable between individuals, depending primarily on individual sizes. Such variability, although not always related to body size, is in keeping with observations of other nudibranch species (Todd,1979a; Todd & Havenhand,1983).

Fig. 5 shows the patterns of total spawn production in relation to maximum post-spawning body size (joules) for nudibranchs in each diet treatment. Although a significant straight line can be fitted to these data, inspection of the figure does indicate a decrease, or perhaps a plateau, in total spawn production for animals larger than approximately 2×10^4 J (10g damp wt). This may be a consequence of the constraints on spawn mass size, for large nudibranchs, alluded to above. More comprehensive data are necessary to clarify this point. Despite the doubts regarding the larger individuals it is apparent that, over most of the size range, there is a clear allometric relationship between body size and total spawn production. Anemone diet, however, does not appear to have any effect on this

Figure 5.

Total spawn production in relation to body size for the nudibranchs feeding on each anemone diet.


Total Spawn Production (J x 10^4)
Maximum Post-Spawning Body J x 10^4
△ = AEQR
□ = AEQG
○ = STRO
△ = MSEN
⊡ = URFE
⊙ = MIXTURE
y = 1.4390x - 0.0325
r = 0.7138, p < 0.001
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5

relationship; animals of equal size, fed on different species, do not produce significantly different quantities of spawn. The effect of diet on reproductive effort (total spawn J/max.post-spawning J as a percentage) was tested using a Kruskal-Wallis non-parametric ANOVA. The results of this test indicate that the null hypothesis of equality of reproductive effort values cannot be rejected ($K = 3.5267$ with 4 degrees of freedom, critical region = 9.488).

DISCUSSION.

Patterns of growth in A.papillosa.

The observed variability in the growth of A.papillosa in this analysis prevents us from making any statements regarding differences in growth of nudibranchs feeding on particular anemone diets. It must be concluded, therefore, that the values of anemone tissues, as food for A.papillosa, do not differ significantly from one another. In order to completely define the relative values of the anemone prey, however, the effects of behavioural and chemical prey defences should be determined.

Perhaps the most interesting feature of these growth data is the marked variation in growth rate between individuals. Other comprehensive data on molluscan growth rates are scant, although information reviewed by Wilbur and Owen (1964) does indicate that large variations in growth rate do occur. For example Cole & Waugh (1959) observed a high proportion of "stunted" oysters

among a commercially cultivated population of "normal" individuals. A proportion of these individuals failed to grow even when transplanted into more favourable conditions. In this particular case, the incidence of the variants may have been attributable to changes in the cultivation technique for the oysters as post-metamorphs (the oyster spat were stored in trays higher on the shore, thus exposing them to more unfavourable physical conditions).

The variability in growth rate shown in Fig. 2 contrasts markedly with published laboratory growth data obtained for other opisthobranch species (Paine,1965; Carefoot,1967; Holleman,1972; Chia & Skeel,1973; Bloom,1974; Todd,1979), in which the molluscs all grew in a consistent manner. There would appear to be three possible explanations for the variability in the present growth data:

i) The results are laboratory artefacts with differential responses among individuals.

ii) Interactions between individuals when they were kept as pairs during the first part of the experiment led to a reduction in the growth rate of some individuals as a result of competition for food. This would not explain why such an effect should continue following the separation of individuals.

iii) The variations represent real, genetic, differences between individuals.

Considering these alternatives in turn: the possibilty that laboratory conditions differentially affect nudibranchs is difficult to assess. It is unlikely, however, that this could account for the majority of the observed variation. This conclusion is drawn on the assumption that all 'weak' genotypes would have been removed from the population by the time the selectively rigorous planktonic and settlement phases have been completed. As a result of this intense selection, the comparatively benign laboratory conditions are unlikely to affect any of the nudibranchs to such a degree. This assumes that individuals with a genetic constitution sufficiently robust for survival in the plankton, and settlement and metamorphosis also possess a correspondingly 'strong' genetic component for post-metamorphic life.

Another possible explanation that is related to the impact of the environment on the genotype is that adverse or stressful conditions may have been experienced by some of the nudibranchs in the short period prior to settlement and that this treatment resulted in a slow growth rate throughout the nudibranchs life. As described previously, this has been suggested to explain incidences of poor growth in oysters (Cole & Waugh,1959).

The second alternative - that competition for food between individuals, in the early part of the experiment, resulted in a decrease in the performance of some individuals - is unlikely. Food was provided to excess at all times and the results of

experiments described in the following chapter show that nudibranchs do not feed at a slower rate when maintained in groups.

The third alternative is a realistic possibility although some features of the data are rather difficult to explain in terms of inherent genetic differences. Of the 48 individuals in the growth experiment, 27 failed to spawn and were, therefore, of zero fitness. Of these, four achieved a maximum size that was greater than the lowest observed for a spawning individual and lived sufficiently long to have reached reproductive maturity. A variety of reasons may explain the absence of spawning in these individuals, but, in view of the size they achieved and their longevity, it is reasonable to suppose that their ultimate zero fitness was not directly related to a low growth rate. For the remaining 23 nudibranchs which grew slowly and did not reach reproductive maturity, one must ask the question: why do individuals with inherent zero fitness persist through generations in such relatively large numbers?.

The studies of Singh & Zouros (1978) and Zouros et al. (1980), provide some valuable insights into this question. In these studies a sample from a cohort of oysters was analysed electrophoretically for seven enzyme loci and the resulting information was related to individual weight. The cohort analysed was one year old, and all the individuals were grown under identical conditions. The analysis showed that individual weight was positively correlated with the number of heterozygous

loci. Several hypotheses can be advanced to explain this observation although it was felt that overdominance for growth rate was the most plausible.

From such observations one might predict that larger A.papillosa are heterozygous at more gene loci. One must then ask the question: why should the number of heterozygous loci - coding for many different enzymes with diverse functions - be positively related to growth rate in this manner?. In accounting for such observations Berger (1976) proposed a molecular scheme whereby heterozygotes may channel a larger proportion of their energy into anabolic functions such as growth and reproduction. From ideas such as this Zouros et al. (1980) concluded that "growth may be one of the few characters affected by a very large number of loci". Thus, its seems likely that growth rate is influenced by a large and diverse complex of genes and that a high degree of heterozygosity among this complex may result in faster growth rates.

If growth is determined in such a manner, it is reasonable to suppose that many combinations of hetero- and homozygous loci are possible. This being the case one might expect growth rate to be highly variable between individuals. In view of the large number of genetic combinations which may result in slow growth, even large individuals may produce a high proportion of slow-growing offspring which do not reach reproductive size.

The underlying causes of variation in individual growth rate within populations are factors of major importance to both geneticists and population ecologists alike. Ecologists in particular tend to screen-out such variability as 'noise' and content themselves with considering the 'average individual'. Clearly, such an approach to ecology is quite inadequate. Of more immediate importance, however, is the question of whether or not the variability observed for A.papillosa is unusual when compared with other species. The contrast between the data presented here and other published laboratory growth data for nudibranchs is notable but the possibility remains that 'noisy' data tend to go unreported. In addition, animals collected from the field for laboratory studies may, in some instances, be selected such that the sample is not representative of the field population. For example, if a species is abundant, collectors are less likely to search the habitat rigorously, and thus, smaller animals are likely to be overlooked. Such biased sampling may result in a lower variability in growth rate in laboratory experiments.

Patterns of reproduction in A.papillosa.

The analysis of reproductive patterns in this species has shown that the anemone prey, as sources of nutrition, confer no differential advantage to individuals in terms of reproductive effort. As with the growth data, this conclusion cannot be extended to include statements regarding the overall prey-value

of intact anemones.

The allometric relationship between energy allocation per spawn mass and body size is to be expected although there is a strong indication from these data that spawn masses produced by the very largest nudibranchs do not exceed those produced by somewhat smaller individuals. This observation is, however, based on only two data points and requires further replication before it can be verified. The apparent plateau in the spawn/body size relationship may represent a physiological constraint, perhaps on the capacity to fertilise mature oocytes with spermatozoa stored from copulation. Detailed histological studies in conjunction with further analysis of the patterns of spawn production may provide a means of verifying such an explanation.

The patterns of spawn production shown by individual nudibranchs were very variable although, in general, there was an increase in the rate of spawning up until the death of the individual. As a general rule larger animals produced larger spawn masses at a faster rate. The increase in rate of spawn production by individuals may be accounted for by the increasing size of the animals over the spawning period - most of the nudibranchs which spawned continued to grow during the spawning period until just before death. De-growth prior to death presumably resulted from the autolysis of somatic tissues and the diversion of the resulting energy into reproductive components (Todd,1978). This change in the energy partitioning of the

system may also account for some of the observed increases in spawn production with the onset of death. The timing of each spawning is likely to be determined jointly by the availability of an adequate number of mature oocytes and stored spermatozoa from a recent copulation. Since, in the present analysis, copulation was permitted on more than one occasion between spawnings it is, perhaps, reasonable to suggest that the observed timing of spawn production was determined by oocyte production.

The patterns of spawn production are very variable between conspecific individuals and nudibranch species in general (Todd & Havenhand,1983). This is illustrated by the contrasting patterns described above for O.muricata and A.proxima (Todd,1979a). The pattern observed in A.papillosa, however, appears to be particularly unusual in that a large proportion of the energy acquired during the reproductive period continues to be diverted towards somatic growth. The observed increases in the rate of spawn production can be accounted for in terms of this increase in body size and as a result of the autolysis of somatic tissues towards the end of the reproductive period. It would appear, therefore, that the patterns of energy allocation between growth and reproduction in this species differ somewhat from other investigated nudibranchs. It should be pointed out, however, that in the nudibranchs as a whole the patterns cover a wide spectrum of alternatives, although most species appear to continue somatic growth for at least a period after the commencement of spawning (Todd & Havenhand,1983).

Chapter 4.

PATTERNS OF CONSUMPTION AND ASSIMILATION IN A.PAPILLOSA (L.)

INTRODUCTION.

The experiments described here concern the consumption rates and assimilation efficiencies for A.papillosa with particular reference to anemone diet. The objective of these experiments was to identify possible correlations between high or low growth rates, on a given anemone diet (as observed in chapter 3), and high or low consumption rates and/or assimilation efficiencies. Clearly, the interpretation of any such correlations would be necessarily tentative and it should be pointed out that these experiments were intended primarily as indicators of possible causal relationships between diet-mediated consumption and/or assimilation patterns and growth. In addition an experiment is described which investigates the effects of group feeding responses on the overall consumption patterns of a single anemone species.

THE EFFECTS OF ANEMONE DIET ON CONSUMPTION RATE.

Inspection of the range of anemone species shows that marked differences exist in the texture and general constitution of their tissues. For example, U.felina is notably more 'leathery' than any of the other species under investigation. On the basis

of this observation one might expect that A.papillosa would consume this species at a slower rate. It was with such possibilities in mind that the experiments described below were initiated.

Two separate experiments have been conducted to investigate the effect of anemone diet on consumption rate. The basic methodology of both experiments was the same although the damp weight measurements of anemone tissues in the first experiment were replaced by weights under water in the second experiment in an attempt to improve weighing accuracy.

MATERIALS AND METHODS.

Consumption Experiment 1 (May-June,1982)

Five anemone species were used in this experiment, each treatment group containing five nudibranchs maintained on the same anemone species throughout the experiment.

The following anemone species were used:

Actinia equina (Red Morph)

Actinia equina (Green Morph)

Sagartia troglodytes

Metridium senile

Urticina felina

Over the experimental period animals were kept in individual plastic mesh cages which were anchored to the bottom of an aquarium tank, and through which seawater constantly flowed. All

animals were starved for 24h before each experiment. On the day of an experiment ten similar sized tissue pieces were cut from individuals of each of the five anemone species. Each piece of tissue, and all of the nudibranchs, were damp-weighed after careful blotting with filter paper. Every effort was made to ensure that each tissue piece was cut from the column of the anemone.

At the start of the experiment all of the nudibranchs in each treatment group were provided with a single piece of pre-weighed anemone tissue of the appropriate anemone species. The remaining five pieces from each anemone were put into identical individual plastic mesh cages to provide controls for any variations in tissue weight that were not attributable to nudibranch feeding.

After 24h all tissue pieces were removed and re-weighed. Changes in the weight of control tissue pieces were subtracted from the corresponding experimental piece to provide a 'corrected' value for the weight of tissue consumed.

The complete experiment was repeated on seven separate occasions and the results were pooled for analysis.

Consumption Experiment 2 (March-April 1983)

This experiment was conducted in a similar manner to experiment 1 with the following exceptions:

i) The green morph of A.equina was not used

ii) Anemone tissue pieces were weighed under water and converted to dry weights for analysis, using species-specific weight under water/dry weight conversion factors. These were obtained from least squares regressions of under water weight(y) on dry weight(x).

iii) Nudibranchs were weighed only at the beginning and the end of the replicate series of experiments.

iv) All five nudibranchs in a diet treatment were individually maintained in a single 2.51 (0.5 gal) plastic container, partitioned into five equal portions and kept in a seawater cascade.

v) The experiment was repeated on five rather than seven occasions.

RESULTS.

Experiment 1.

Table 7 shows the animal weights and 'corrected' amounts of tissue consumed in twenty-four hours for the seven replicates of the experiment. Mollusc weight was very variable, as much as doubling over the seven replicates in some cases (e.g. mollusc

Table 7. Mollusc damp wt(g) and corrected damp wt of tissue consumed $24h^{-1}$

		(12.02.82)		(15.02.82)		(19.02.82)		(22.02.82)		(26.02.82)		(01.03.82)		(05.03.82)	
		Mollusc Damp Wt	Corrected Damp Wt Eaten	Mollusc Damp Wt	Corrected Damp Wt Eaten	Mollusc Damp Wt	Corrected Damp Wt Eaten	Mollusc Damp Wt	Corrected Damp Wt Eaten	Mollusc Damp Wt	Corrected Damp Wt Eaten	Mollusc Damp Wt	Corrected Damp Wt Eaten	Mollusc Damp Wt	Corrected Damp Wt Eaten
AEQR	1	0.930	0.0443	1.097	0.0387	1.305	0.0481	1.005	0.0848	1.157	0.0822	1.317	0.1512*	1.535	0.0687
	2	1.372	0.0487	1.427	0.0777	1.870	0.0823	1.473	0.0601	1.610	0.0296	1.725	0.0368	1.946	0.0401
	3	0.577	0.0996	0.700	0.0194	0.834	0.0819	0.745	0.0458	0.945	0.0640	1.150	0.0466	1.214	0.0441
	4	0.534	0.0510	1.427	0.0079	0.566	0.0470	0.595	0.0311	0.730	0.0581	0.941	0.0161	0.972	0.0779
	5	0.481	0.0709	1.097	0.0452	0.624	0.0667	0.643	0.0336	0.733	0.0757	0.945	0.0283	0.968	0.0820
AEQG	1	0.850	0.0687	0.903	0.0633	1.119	0.0134	0.972	0.1102	1.063	0.1289	1.337	0.0196	1.371	0.0508
	2	0.775	0.0656	0.792	0.0649	0.843	0.0558	0.792	0.0605	0.906	0.0419	1.091	0.0370	1.212	0.0760
	3	0.530	0.0528	0.637	0.0748	0.646	0.0739	0.677	0.0500	0.778	0.0785	0.899	0.0421	0.986	0.0564
	4	0.866	0.0925	1.011	0.0975	1.108	0.0727	1.091	0.0667	1.183	0.0416	1.324	0.0171	1.408	0.0539
	5	0.713	0.0578	0.807	0.0346	0.947	0.0644	0.870	+0.0047	0.993	0.0559	1.127	0.0585	1.340	0.0417
STRO	1	1.395	0.0970	1.127	0.0978	1.317	0.0396	1.217	0.0822	1.390	0.1369	1.588	0.1328	1.817	0.1042
	2	1.196	0.0984	1.122	0.1135	1.280	0.0767	1.142	0.1040	1.260	0.1164	1.496	0.1376	1.784	0.0933
	3	1.412	0.0713	1.569	0.1174	1.780	0.0021	1.449	0.0445	1.569	0.0850	1.729	0.0531	1.933	0.0031
	4	1.081	0.1034	1.319	0.1156	1.565	+0.0932*	1.208	0.1141	1.385	0.0584	1.552	0.0713	1.854	0.0616
	5	0.560	0.0831	0.051	0.0846	0.936	0.0182	0.668	0.0623	0.749	0.0742	0.990	0.0858	1.105	0.0273
MSEN	1	0.625	0.0145	0.705	0.0148	0.728	+0.0012	0.556	0.0043	0.528	0.0179	0.650	0.0367	0.551	0.0161
	2	0.447	0.0644	0.465	0.0623	0.584	0.0361	0.510	0.0640	0.522	0.0722	0.688	0.0558	0.769	0.0953
	3	0.558	0.0397	0.555	0.0377	0.695	0.0407	0.620	0.0579	0.693	0.0568	0.875	0.0441	0.893	0.2304*
	4	0.473	0.0231	0.505	0.0029	0.539	0.1091	0.525	0.0812	0.550	0.0526	0.770	0.0178	0.826	+0.0048
	5	0.272	0.0685	0.304	0.0039	0.392	0.0227	0.320	0.0365	0.368	0.0664	0.480	0.0186	0.519	0.0415
URFE	1	1.387	0.0549	1.420	0.1044	1.637	0.0623	1.416	0.0935	1.646	0.0332	1.840	0.0671	2.078	0.0637
	2	1.750	0.0219	1.098	0.0879	1.323	0.0585	1.104	0.0365	1.298	0.0353	1.651	0.0622	1.744	0.0455
	3	1.187	+0.0087	1.378	0.0753	1.497	0.0216	1.247	0.0454	1.472	0.0466	1.544	0.0469	1.803	0.0350
	4	1.330	0.0431	0.928	0.0804	1.153	0.0713	0.953	0.0755	1.085	0.0735	1.256	0.0314	1.465	0.0422
	5	0.654	0.0183	0.688	0.0635	0.813	0.0514	0.791	0.0471	0.949	0.0538	1.102	0.0117	1.199	0.0511

* Denotes suspected errors in weight recordings.

three in the red A.equina treatment), and falling by up to one third between two observations (e.g. 12th - 15th February for mollusc two in the U.felina treatment). The change in weight of the control anemone tissue pieces was less than 0.05g for all but three readings: mollusc 3 (replicate 7) feeding on M.senile; mollusc 4 (replicate 3) feeding on S.troglodytes, and mollusc 1 (replicate 6) feeding on red A.equina. The changes in control tissue weight for these data points were +0.1143g, -0.1161g and +0.073g respectively. In view of the magnitude of change in these readings, compared to all others, it seems likely that an error was made in recording these weights (possibly by 0.1g in each case). The data set has, therefore, been analysed both including and excluding these data.

Table 8 shows the mean consumption rates, and their associated standard errors, for each treatment, with and without the suspected data. The two values of Cochran's test statistic shown at the bottom of the table both indicate that there is a marked departure from the null hypothesis of homogeneous variances (see Underwood,1982). Thus, one of the assumptions of a parametric ANOVA is violated. Log-transformation of these data does not improve this situation. In view of this a non-parametric single-factor ANOVA (the Kruskal-Wallis test) was performed. If a parametric test had been permitted for these data, a two-factor ANOVA would have been the most appropriate with both mollusc weight and diet treatment as factors. There is, unfortunately, no non-parametric equivalent to the two-factor ANOVA and thus, mollusc weight could not be included in the

Table 8. Mean consumption rates for complete and truncated data sets in feeding experiment 1 (±1 s.e.)

Diet	Complete Data Set Mean Consumption Rate ($g\ eaten\ 24h^{-1}$)	Truncated Data Set Mean Consumption Rate ($g\ eaten\ 24h^{-1}$)
AEQR	0.0567 (±0.00467)	0.0540 (±0.00386)
AEQG	0.0582 (±0.00446)	0.0582 (±0.00446)
STRO	0.0764 (±0.00776)	0.0814 (±0.00612)
MSEN	0.0442 (±0.00719)	0.0471 (±0.00725)
URFE	0.0515 (±0.00404)	0.0515 (±0.00404)
Cochran's Test Statistic	0.3542**	0.37004**

Asterisks denote conventional significance level.

model. Clearly this factor may affect consumption rates and the consequences of its exclusion from the analysis are discussed further below.

The outcome of the Kruskal-Wallis test is the rejection of the null hypothesis of equality of consumption rates between all or some of the diet treatments ($K = 26.54799$, $p < 0.001$, critical value = 18.467, for the truncated data set). This outcome remains unchanged if the complete data set is used. In order to determine which diet treatments differ significantly from one another, a set of ten Mann-Whitney U-tests were performed between all pairs of treatments.

Table 9 shows the results of these tests for the truncated data - the outcome using the complete data set is the same. The results show that M.senile was consumed at a significantly slower rate than S.troglodytes, red A.equina or green A.equina. Furthermore, S.troglodytes was consumed at a faster rate than U.felina. This outcome is somewhat inconclusive, perhaps for two major reasons. First, it was felt that the damp-weighing procedure might have incurred gravimetric errors and secondly, there was, perhaps, inadequate replication. The most probable pattern, however, is that S.troglodytes = green A.equina = red A.equina > U.felina = M.senile. It was in view of this inconclusive result that experiment 2 was conducted.

Table 9. The results Mann-Whitney U-tests between all pairs of means for feeding experiment 1

Calculated Values of the Mann-Whitney U-statistic

AEQG	665			
STRO	874	866		
MSEN	423.5*	425*	242.5***	
URFE	562.5	519.5	292.5***	705
	AEQR	AEQG	STRO	MSEN

Asterisks denote conventional significance level.

Experiment 2.

Table 10 shows the dry weights of anemone tissue consumed in twenty four hours, predicted from weights under water using species-specific wet/dry weight conversion factors.

For the initial analysis the data for all replicates of each diet treatment were pooled and a single-factor ANOVA was performed. Cochran's test for non-homogeneity of variances was performed prior to the ANOVA and no marked departure from homogeneity of variances was detected ($C = 0.3073$, critical value $= 0.400$, $p=0.05$ with $K=4$ treaments and 25 d.f.).

Table 11 shows the mean consumption rates for each diet and the results of the single factor ANOVA. This test indicates that the null hypothesis of equality of consumption rates on each anemone diet cannot be rejected. The assumption of normal distribution of error terms was not tested but no marked departure from normality was apparent; the example of Underwood (1982) is followed in this respect, who concludes that "it seems safe to disregard the assumption of normality unless very gross violations are actually evident in the data". Examination of the experimental design shows that samples were independently drawn.

Table 10. Corrected dry weights of tissue consumed $24h^{-1}$ in feeding experiment 2.

Diet	Mollusc Number	Replicate Number (with Date) 1 18.03.83	2 21.03.83	3 30.03.83	4 08.04.83	5 14.04.83
AEQR	1	63.5*	----	83.5	119.9	96.0*
	2	25.3	39.5	21.5	42.0	105.9
	3	41.0	30.5	40.0	73.5	71.5
	4	1.5	+4.5	+6.5	16.0	17.0
	5	25.5	----	22.5	37.0	42.5
STRO	1	44.8*	50.1*	20.7	3.1	100.2*
	2	36.9	30.8	79.1*	40.9	90.6*
	3	45.7	41.3	+0.4	32.5*	65.9*
	4	29.5	1.7	15.8	27.7*	53.6*
	5	17.6	11.4	1.7	16.7	40.9
MSEN	1	40.6*	61.5	89.8*	71.1*	114.0*
	2	+8.5	17.5	13.0	0.6	65.5
	3	+4.5	+11.8	20.3	24.8	45.7
	4	23.7	0.0	+13.0	0.6	65.5
	5	1.7	+11.8	9.6	11.8*	45.2
URFE	1	20.3	70.5	19.7	67.5	75.3
	2	16.1	43.6	41.8	103.4	36.5
	3	3.6	31.1	37.0	53.2	41.2
	4	11.9	----	54.4	39.4	50.2
	5	+3.6	----	1.8	21.5	23.9

\+ Denotes an increase in corrected tissue weight
* Denotes complete consumption of tissue before the end of the experiment

Table 11. Group Means, Variances, and the results of a Single-Factor ANOVA for feeding experiment 2.

Diet Treatment	Mean Consumpton (mg Dry Wt)	Variance
AEQR	43.7	1182.62
STRO	35.952	728.352
MSEN	25.624	1147.285
URFE	37.4043	674.950

Cochran's Test Statistic = 1147.285 / 3733.207
= 0.3037 N.S. (Critical Value 0.3720)

No marked departure from homogeneity of variances is detected

Scource of Variation	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F-ratio
Among Treatments	4.12×10^3	3	1.376×10^3	1.40 N.S.
Within Treatments	9.04×10^4	92	9.826×10^2	
Total	9.452×10^4	95		

Table 12 shows the results of a two-factor analysis of covariance performed by Dr A.D.Gordon, Dept of Statistics, University of St.Andrews, with diet treatment and temperature as factors and mollusc initial and final weights as co-variates. The results of this analysis once again indicate that there are no differences in the consumption rates for nudibranchs feeding on different anemone diets. It can be seen from Table 10, however, that in nine out of the 25 values obtained for consumption on S.troglodytes, the tissue was completely eaten at the end of the experiment. Thus, the consumption values for this species are likely to have been under-estimated.

The results of experiments 1 and 2 leave conclusions regarding the rate of consumption of anemone species somewhat equivocal. In view of the conflicting results between the two experiments, however, it is clear that any differences which do obtain are not of great magnitude. On the basis of these results, therefore, it would appear that species-specific rates of consumption are unlikely to be important factors in the decision rules which are expected to govern A.papillosa predation.

DISCUSSION.

The objective of the above experiments was to detect differences in the consumption rates of nudibranchs in relation to anemone diet. Although the pattern which emerges from the results is

Table 12. Results of a Two-Factor Analysis of Covariance (diet and temperature as factors, initial and final mollusc weight as co-variates) for experiment 2.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Sum of Squares	F-ratio
Between Groups (with co-variates)	8.3×10^2	3	2.766×10^2	0.71 N.S
Within Groups (with co-variates)	3.3084×10^4	86	3.847×10^2	
Total	3.391×10^4	89		

H_o : All anemone diets are consumed at equal rates - cannot be rejected

somewhat equivocal, it is apparent that the magnitude of any differences which do obtain are small and often obscured by an overall variability in consumption rates. Thus, it is reasonable to conclude that the textural properties of the anemone tissues used in these experiments do not have a differential effect on the ability of nudibranchs to consume them.

In view of the possible underestimation of S.troglodytes consumption in experiment 2, and the high position of this species in experiment 1, the possibility remains that this species is consumed at a significantly faster rate than the tissues of other anemone species. In view of its small size, however, any energetic advantage that may accrue from such an ability is likely to be balanced by the necessarily frequent investment of time and/or energy in seeking-out another prey item. Furthermore, the calorific analyses described in chapter 2 indicate that S.troglodytes is, in gross calorific terms, the least valuable of all the species studied.

The inability to include mollusc weight in the statistical model for experiment 1 is likely to have contributed to the difficulties in the interpretation of these results. Table 7 shows that molluscs in the M.senile treatment group were somewhat smaller than those in the other groups. This factor may have contributed to the lower consumption rate observed on this species. Including mollusc weight as a co-variate in experiment 2 did improve the model's description of the data, thus supporting the hypothesis that consumption rate is size-related.

The data collected in these experiments are, however, too variable to define a clear mollusc size-consumption rate relationship. Indeed, this variability may, in itself, be an important factor in the overall consumption patterns of this species and may indicate the absence of any stong selection pressure to maximise the rate of tissue intake. Clearly, these experiments have only effected a test for differences in the rate of consumption of anemone tissues; they take no account of the defensive and/or escape responses of intact prey items. On the basis of these results, therefore, it is reasonable to suggest that any differences in consumption rate that are detected between intact anemone species are attributable to behavioural factors and chemical defensive responses that were obviated by the presentation of cut tissue pieces.

Experiments on species-specific consumption rates for whole anemones have not been attempted. The variations in consumption rates between replicates and individuals in the above experiments are certain to be increased considerably for intact prey individuals. Furthermore, size differences between anemone species would involve yet another variable to be resolved; the replication required to sufficiently resolve the patterns of consumption with intact anemones was, therefore, considered prohibitive within the context of this study.

THE EFFECT OF GROUP FEEDING ON CONSUMPTION RATE IN A.PAPILLOSA (L.)

It has long been noted that A.papillosa are more strongly attracted to damaged or wounded anemones (Braams & Geelen,1953). The present experiment, however, was prompted by the observation that nudibranchs maintained in groups often feed together on a single cut piece of anemone tissue when more than one cut piece was available. The present experiment was, therefore, designed in order to ascertain whether individual net consumption rate was decreased (by interference) or (for as yet uncertain reasons) increased. It is possible, for example, that individuals attempt to out-compete others by sharing an item despite the presence of other available prey.

MATERIALS AND METHODS.

Throughout this experiment, two groups (A and B) of five similarly sized nudibranchs were maintained on the red morph of A.equina since this was the most available species. All animals were starved for the 24h preceding each replicate experiment. In each replicate the nudibranchs of one group were maintained as separate individuals and provided with a single pre-weighed piece of A.equina. The nudibranchs in the second group were not separated and were provided with a single large piece of pre-weighed A.equina. Appropriately sized control pieces were used to measure any weight changes that were not a consequence of feeding. All tissue pieces were weighed under water and

converted to dry weights for analysis using the conversion factor obtained in experiment 2 above.

In the succeeding replicate experiment the two groups were swapped so that the nudibranchs which were previously grouped were now kept as individuals and vice versa. The experiment was repeated ten times so that each group was tested both as individuals and as a group on five occasions. The results for individuals and groups were pooled for the analysis.

RESULTS.

Table 13 presents the corrected weights of red A.equina consumed in the experiments. Examination of these data shows that, in seven of the ten comparisons of 'individual' versus 'group' consumption, separated individuals consumed more food. The magnitude of difference was very variable and on the basis of these results there is no evidence for any number-mediated consumption effects. The raw data are sufficiently clear to render formal statistical analysis unnecessary.

DISCUSSION.

The results of this experiment clearly do not support the hypothesis that group feeding responses result in an overall decrease in the rate of food consumption. Neither do they indicate any increase in consumption rate as a result of co-operative effects between individuals.

Table 13. Corrected dry weights(mg) of A.equina (red morph) consumed $24h^{-1}$ for nudibranchs as individuals and in groups

Group A

Replicate No	1	2	3	4	5
			Individual Consumption		
Date	19.04.83	30.04.83	17.05.83	24.05.83	10.06.83
Temp°C	6.0	8.0	8.7	10.9	11.0
	21.4	9.9	124.4	35.3	82.1
	23.9	22.9	63.7	45.3	35.8
	19.9	25.4	39.3	58.7	44.8
	17.4	15.4	11.4	+44.3	59.7
	23.4	19.9	48.7	+0.5	+3.0
	106.0	109.4	287.5	94.5	222.4
			Group Consumption		
Date	26.04.83	04.05.83	21.05.83	29.05.83	13.06.83
Temp°C	7.2	7.6	9.7	9.2	10.0
	105.5	48.7	99.0	254.7	140.3

Group B

Replicate No	1	2	3	4	5
			Individual Consumption		
Date	26.04.83	04.05.83	21.05.83	29.05.83	13.06.83
Temp°C	7.2	7.6	9.7	9.2	10.0
	8.9	18.9	34.8	61.7	65.7
	+0.5	9.9	55.2	39.3	27.8
	13.4	4.5	40.8	20.4	28.8
	5.5	5.5	20.9	82.1	55.2
	3.0	16.9	21.9	20.4	42.3
	30.3	55.7	173.6	223.9	219.8
			Group Consumption		
Date	19.04.83	30.04.83	17.05.83	24.05.83	10.06.83
Temp°C	6.0	8.0	8.7	10.9	11.0
	33.8	83.9	142.3	89.0	104.5

This outcome only holds with respect to tissue pieces, that is, from the point at which the intact anemones' defences have been overcome. It is likely that nudibranchs are attracted to damaged anemones or those upon which other nudibranchs are feeding because the 'costs' of overcoming defensive adaptations are reduced when attacking an already partially disabled prey. Since group feeding has no effect on consumption rate after the anemone is subdued, such a hypothesis would be consistent with the results of the above experiments. Detailed and extensive observation of attacks by individuals and groups of nudibranchs would, however, be required before such a hypothesis could be substantiated.

THE EFFECTS OF ANEMONE DIET ON ASSIMILATION EFFICIENCY

In this study assimilation efficiencies have been gravimetrically determined by comparing the inorganic ash component of the food consumed and the faeces produced. Assimilation Efficiency is given by the ratio:

$$\frac{\phi F - \phi C}{\phi F(1 - \phi C)}$$

where: ϕC = the weight fraction of ash in the food (anemone) consumed - given as % inorganic ash in table 3, and

ϕF = the weight fraction of ash in the faeces (see Crisp,1971).

It should be noted that assimilation efficiency can be obtained without measuring either the rates of ingestion or egestion. This method has been used by Conover (1966) to measure the assimilation efficiencies of copepods, and by Carefoot (1967) to measure food consumption in the nudibranch Archidoris pseudoargus (Rapp).

MATERIALS AND METHODS.

The inorganic ash fractions of the anemone species were presented in Table 3, and are used in the calculations here. The inorganic ash fraction of the faeces of animals feeding on different anemone diets were obtained from collected faecal material produced by the nudibranchs from the growth experiments described in chapter 3.

Faeces were easily identifiable in the experimental cages and could be drawn into a pipette and dropped into 0.9% Ammonium Formate to remove surface salts. After washing in this manner the faeces were placed on individual, pre-weighed aluminium pans and freeze-dried. The inorganic ash fraction was then determined in the standard manner after incineration in a muffle furnace at 550^{o}C for 4h. Fifteen replicate ash determinations were obtained for animals feeding on each of the anemone diets, with the exception of M.senile-maintained animals, from which only eight faeces could be collected. Only clearly identifiable, integrated, faeces were chosen for analysis, all of which were collected within 24h of production. Carefoot (1967) compared the concentration of nitrogen in fresh faeces from the opisthobranch Aplysia punctata (Cuvier) with faeces which had been in seawater at 15^{o}C for 12, 24 and 48h since excretion. The total loss of nitrogen over 48h was only 3% of the original concentration, a value well within the variability inherent in the analysis. It is unlikely that such errors, associated with the bacterial

degradation of faeces, are any greater than 3% in the present study.

Owing to the relative difficulty in obtaining quantities of clearly identifiable faeces, no account was taken of individual variations in assimilation efficiency.

RESULTS.

Table 14 shows the assimilation efficiencies (expressed as a percentage) calculated for individual faeces from animals feeding on different anemone diets. To detect differences between the mean assimilation values for the five diet treatments a single-factor ANOVA would normally be appropriate. Before such an analysis could be performed on these data, however, the individual assimilation efficiency values require arc-sine transformation. This is because the assimilation efficiencies are expressed as proportions or percentages and the variances will decrease for samples with means lying closer to the boundaries at zero or unity (Underwood,1981). Using Cochran's Test for these transformed data, the null hypothesis of homogeneity of variances is convincingly rejected ($C = 0.4985$, $p < 0.001$, critical value = 0.4000) and thus, one of the assumptions of an ANOVA is violated. In view of this the non-parametric ANOVA, the Kruskal-Wallis Test, was applied. This test makes no assumptions concerning the normality of the parent distribution or of the homogeneity of the sample variances. The result of this analysis is to convincingly reject the null

Table 14. Assimilation Efficiencies for individual faeces from molluscs maintained on different anemone diets.

Diet	AEQR	AEQG	STRO	MSEN	URFE
	62.88	66.96	88.76	72.78	89.43
	88.81	75.51	86.05	76.51	72.69
	89.85	88.38	93.39	73.53	84.96
	71.56	94.47	92.94	79.54	82.46
	69.22	83.23	86.38	62.21	56.85
	86.79	89.24	90.22	77.89	76.93
	70.56	65.47	88.82	60.99	78.11
	67.93	87.29	80.13	83.85	35.35
	88.82	89.21	83.94	-----	85.82
	84.53	62.59	91.05	-----	77.94
	88.19	87.24	89.72	-----	21.35
	71.82	81.52	90.00	-----	58.20
	93.39	78.91	85.78	-----	76.54
	86.83	87.94	95.59	-----	75.58
	76.05	92.35	94.09	-----	56.05
Mean A.E.	79.81	82.02	89.12	73.41	68.55
s.e.	2.50	2.52	1.03	2.67	4.88

Cochrans Test Statistic for Arc-Sine transformed counts.
$C = 0.4985$ $p < 0.001$

H_o convincingly rejected - transformed variances are not homogeneous, thus violating one of the assumptions for a one-factor ANOVA.

hypothesis of equality of means ($K = 23.467$, $p < 0.001$, critical value = 18.467). In order to determine which means differ significantly, Mann-Whitney U-Tests were performed on all pairs of means. The results of the Mann-Whitney U-Tests are shown in Table 15.

The results presented in Table 15 show that the assimilation efficiency for nudibranchs feeding on S.troglodytes is significantly greater than for nudibranchs feeding on any other species. The situation with regard to the two colour morphs of A.equina is somewhat less clear; assimilation of green A.equina is indicated as being significantly greater than for either U.felina or M.senile, while no difference can be detected between red A.equina and these two species. Furthermore, no significant difference can be detected between the red and green morphs of A.equina. More replication is evidently required before any firm conclusion could be drawn about the assimilation of these species. It is likely, however, that both the colour morphs of A.equina are assimilated more efficiently than either U.felina or M.senile i.e. S.troglodytes > A.equina (green) = A.equina (red) > M.senile = U.felina.

DISCUSSION.

The assimilation efficiencies calculated from the results of this experiment are close to those obtained by Carefoot (1967) for Aplysia punctata (67%) and Dendronotus frondosus (Ascanius) (86%) using a similar index. Paine (1965), however, reports

Table 15. The results of Mann-Whitney U-Tests between all pairs of means for the assimilation efficiency of molluscs maintained different anemone diets.

	Calculated Values of the U Statistic			
AEQG	100			
STRO	51 **	61*		
MSEN	40	26*	1**	
URFE	76	56 *	13 **	61
	AEQR	AEQG	STRO	MSEN

Asterisks denote conventional significance level.

somewhat lower values (usually between 50% and 70%) for the bullomorph Navanax inermis (Cooper), using a different and more detailed methodology.

In the context of the objectives of this study, the differences in assimilation efficiency between anemone diets is rather difficult to interpret. The results of the growth experiments reported in Chapter 3 failed to detect any significant diet-related differences in the performance of nudibranchs. Thus, the apparent differences in assimilation efficiency are not reflected in differences in overall growth and reproduction. This may be because feeding on diets with lower assimilation efficiencies is counterbalanced by increased consumption, thus producing similar growth efficiencies on all diets. The experiments in the previous section of this chapter, however, do not indicate that this is the case. Another possible reason for the absence of any correlation between the observed assimilation efficiencies and growth and reproduction is that specific nutritional characteristics of the anemone species, which do not correlate with assimilation efficiency, negate the expected advantage of preying on an easily assimilated diet.

One notable outcome from the growth experiments was that individual variations in growth rate were considerable. Although the present observations have not taken specific account of variations in assimilation efficiency between individuals, one would expect that differences in the individual assimilation efficiencies of molluscs within a treament group would be

reflected in the variations in the assimilation of the collected faeces. Thus, large variances for each diet treatment should indicate a high degree of individual variability. Examination of Table 14 shows that this is cleary not so - the largest standard error about the mean is only 4.8% (U.felina). These results do not indicate, therefore, that the variations in individual growth rates described in Chapter 3 are attributable to variations in the assimilation efficiencies between individuals.

Of all the anemone species examined in the foregoing experiments S.troglodytes stands out for a number of reasons. In terms of its gross calorific value S.troglodytes is notably lower than the other four species. On a proportional basis its gross biochemical composition, however, is not markedly different from the other species although it should be emphasised that only 67% of the total ash-free dry weight was accounted for by the analyses (see Table 2). In the first consumption experiment there was an indication that S.troglodytes might be consumed at a higher level, furthermore, in the second consumption experiment it is likely that consumption rates on this species were underestimated. Finally the assimilation efficiency for S.troglodytes was shown to be significantly greater than for the remainder.

The indications are, therefore, that S.troglodytes might possibly be a preferred item amongst the studied species and this is, perhaps, substantiated by the preponderance of field associations of A.papillosa with S.troglodytes in the intertidal

at both Robin Hood's Bay and St Andrews. Admittedly, more extensive field observations around the British Isles are required.

The subsequent behavioural experiments have, therefore, been designed especially with regard to S.troglodytes and A.equina (a frequently cited preferred species).

Chapter 5.

THE EFFECTS OF MAINTENANCE DIET ON PREY-SPECIES SELECTION

INTRODUCTION.

A.papillosa has been the subject of a number of behavioural and autecological studies in which particular emphasis has been laid upon specific preferences for anemone prey-species. The methodologies employed to investigate prey preferences have been diverse and, in most cases, a preference for one of several species was shown (e.g. Stehouwer,1952; Braams & Geelen,1953; Robson,1961; Waters,1973; Edmunds et al.,1974,1976).

In early laboratory studies both Stehouwer (1952), and Braams & Geelen (1953), utilised a variant of the standard Y-maze in which groups of nudibranchs demonstrated their preference by crawling along one or other arm into a chamber containing an anemone species. Prey preference was determined from the numbers found in each anemone chamber after a given period. Braams & Geelen (1953) concluded that A.papillosa showed equal preference for A.equina and M.senile while the work of Stehouwer (1952) indicated a preference for M.senile. A.equina, however, was not included in the latter study. More recently Tardy & Bordes (1978) have utilised a similar Y-maze type apparatus in which A.papillosa demonstrated a marked preference for Anthopleura balli (Cocks).

Both Miller (1961) and Swennen (1961) assessed prey-preferences from feeding observations where the nudibranch was presented with single anemone species in glass dishes. Both of these studies indicated a preference for A.equina. A variant of this approach was adopted by Waters (1973) who determined prey-preferences for Californian A.papillosa from the results of two different experiments. In the first of these, individuals of five anemone species were maintained in laboratory aquaria with groups of nudibranchs; preference was determined from the order in which species were consumed. In the second behavioural experiment pairs of anemone species were presented to groups of nudibranchs. The results of Waters' studies indicated a preference for Epiactis prolifera (Verrill), Anthopleura elegantissima (Brandt) and Anthopleura xanthogrammica (Brandt).

Edmunds et al. (1974), in behavioural experiments with both European and Californian anemones, used a combination of paired and single presentations of prey items to individual nudibranchs. A.equina, A.elegantissima and Anemonia viridis (=sulcata) were indicated as being the preferred species. Yet other laboratory observations by Wolter (1967) (Helgoland) and Robson (1967) (Denmark) have indicated preferences for M.senile and Stomphia coccinea (Müller) respectively.

In view the wide geographic distribution of A.papillosa - and the corresponding differential availability of prey anemone species - some large-scale differences in predator behaviour are to be expected; however, the laboratory studies outlined above indicate apparent discrepancies in feeding behaviour even within given localities. The few reported field observations from N.W. Europe have contributed little to clarify the laboratory data, with large populations of A.papillosa noted in association with M.senile (Gorzula & Cameron,1976), Cereus pedunculatus (Pennant) (Tardy, quoted by Harris & Howe,1979) and S.troglodytes (Todd,1981). M.senile is almost exclusively sublittoral, while the S.troglodytes-associated populations cited above and exploited in the present study were strictly intertidal. The possibilities of differential behaviour and responses of sublittoral and intertidal A.papillosa in the same locality must, therefore, be considered. Nevertheless, one might expect rather more consistent prey-preferences within a given locality than the previous studies of A.papillosa suggest.

One factor which may play a major role in explaining the variability of A.papillosa prey preferences alluded to above concerns dietary experience and the possibility of (prey) frequency-dependent modifications of preference. This type of predatory activity, or "switching" behaviour (sensu Murdoch,1969; Murdoch & Oaten,1975; Murdoch et al.,1975), implies the taking of disproportionate numbers of the most abundant (but not necessarily most acceptable) prey item when prey preference is

weak. In this context, McNair (1980, 1981), in particular, has recently considered the influence of "training effects" (such as "search image" formation) on the composition of a model predators' diet within the premises of optimal foraging theory. One of the principal conclusions of these theoretical developments is that training effects may result in switching responses (McNair, 1980). Such might be the case for A.papillosa. Harris (1973) and Waters (1973) have hinted at the possible effects of previous dietary experience of A.papillosa in laboratory tests although its importance remains unestablished.

"Ingestive conditioning" (Wood,1968) concerns the modification and/or reinforcement of prey preferences, and hence responsiveness to prey-related chemicals, by recent feeding experience. This has been demonstrated for a wide range of invertebrates including Platyhelminthes, Crustacea, Mollusca and Echinodermata (see Sloan,1980 for review). With particular reference to A.papillosa Harris (1973) noted that both Stehouwer (1952) and Braams & Geelen (1953) had demonstrated a preference for M.senile in nudibranchs collected in association with sublittoral clones of this species. Furthermore, A.papillosa from the east coast of the U.S.A. have been shown to choose their 'normal' prey species - M.senile - over Anthopleura elegantissima, which is the most preferred item for Californian A.papillosa (Waters and Pelletier, quoted by Harris 1973). By contrast, Waters (1973) discounted the importance of ingestive conditioning in determining preference for A.elegantissima over A.xanthogrammica and E.prolifera by A.papillosa; this was despite

the fact that A.elegantissima was the primary associate in the field. The same author does, however, report possible evidence for conditioning on Urticina (=Tealia) coriacea (Cuvier) and U.crassicornis (Müller) and also cites additional observations of reversible conditioning to M.senile and E.prolifera respectively.

It was in view of these possibilities that the behavioural experiments reported below were undertaken. Specifically, prey preference studies have been conducted under controlled laboratory conditions with nudibranchs collected in the field and of known dietary history. For behavioural experiments performed in the U.K. animals had been exclusively associated with intertidal S.troglodytes and in this respect provided a fixed starting point from which dietary experience could be manipulated. In the first behavioural experiment a prey-preference hierarchy has been elucidated for A.papillosa maintained on the field diet (S.troglodytes): subsequently the hierarchy was re-established after maintaining the same nudibranchs for a period on an apparently little-preferred species (the red morph of A.equina). In the light of these results two further behavioural experiments were performed, for both U.K. and N.W Pacific A.papillosa, in which the effects of two-stage changes in diet were investigated.

BEHAVIORAL EXPERIMENT 1

MATERIALS AND METHODS.

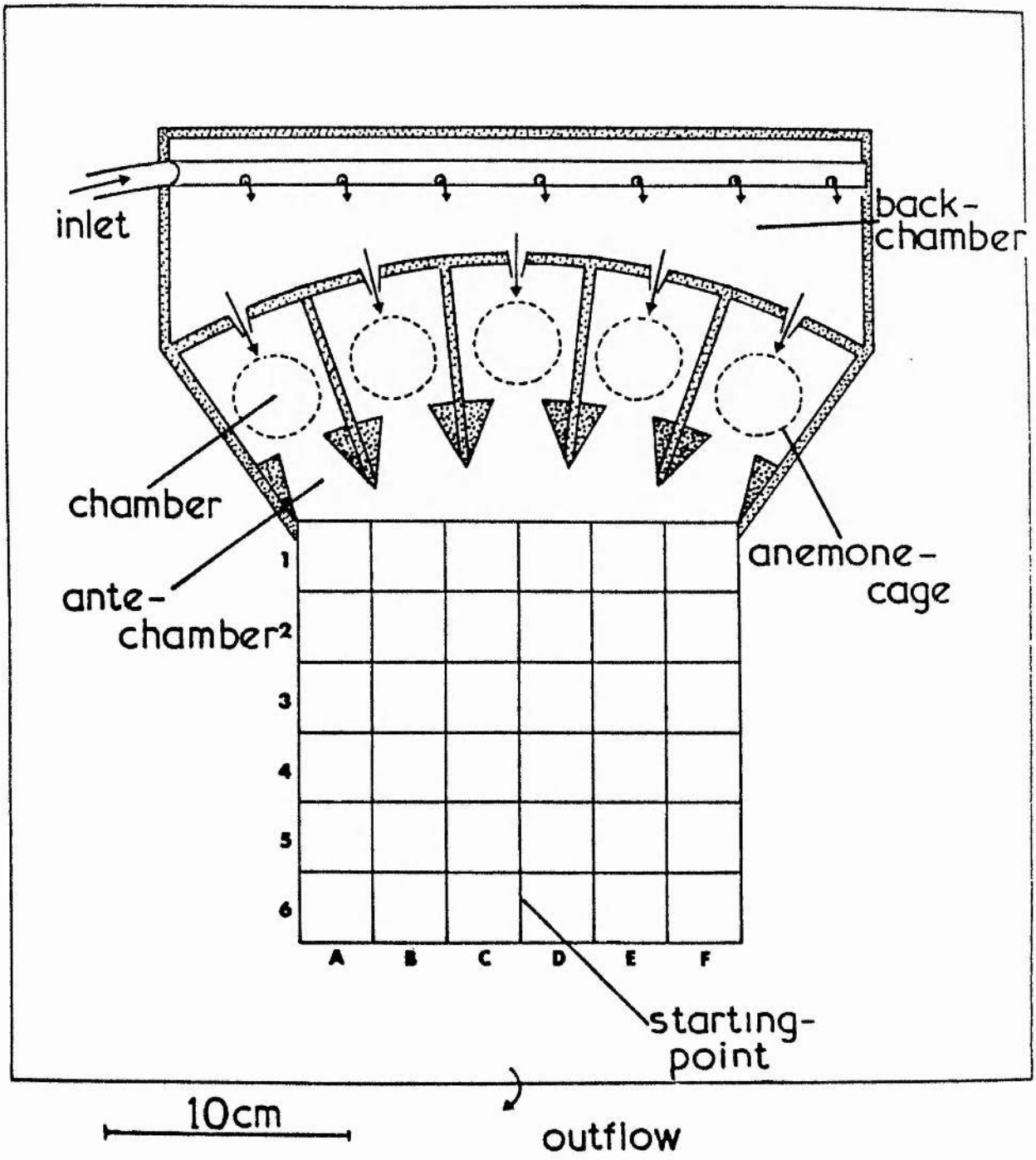
Experiments were conducted in the laboratory between March and August 1981 with 21 nudibranchs collected intertidally at Robin Hood's Bay, North Yorkshire. Each nudibranch was maintained in a separate cage throughout the experimental period thereby allowing individual performances to be recorded.

A.papillosa undoubtedly detects prey in a chemosensory manner (Braams & Geelen,1953; Swennen,1961; Edmunds et al.,1974) and will orient into low velocity currents when the water source includes a suitable prey item (Haaften & Verwey,1960). The objective of this behavioural experiment was to offer a range of prey species simultaneously in a multi-chamber flow apparatus and to elucidate a prey-preference hierarchy on the basis of chamber selection alone. No reinforcement of the prey selection (by feeding) could be permitted since this would obviously upset control of the maintenance dietary regime.

Fig. 6 shows the chamber apparatus comprising of five contiguous, radially-arranged chambers within which the individual live prey anemones could be placed. Each chamber had a restricted opening of 1.5cm which formed an 'ante-chamber' and all five chambers were supplied with seawater from a single back chamber. Anemones were held in small weighted plastic mesh cages

Figure 6.

Diagram showing multi-choice chamber apparatus.


inlet
back-
chamber
chamber
ante-
chamber
anemone-
cage
1
2
3
4
5
6
A
B
C
D
E
F
starting-
point
10cm
outflow

in order both to facilitate operator handling of the prey and cleaning of the apparatus, and to prevent the nudibranchs from feeding having once made a selection. Flow of seawater through the five chambers was similar in all cases and equitability was enhanced by the inclusion of glass nozzles at the back of each chamber and by providing a dispersed input through a drilled tube. This minimised vorticity in the back chamber. The outflows from the five chambers converged approximately 18cm from the openings and it was at this point that the nudibranch was introduced. The rate of water flow through each choice apparatus was maintained constant within and between trials by supplying seawater from a constant header tank system. Preliminary dye flow trials had also shown an even flow of water through each individual chamber, with flow rates ranging from 95-106ml min^{-1} for each. An outflow siphon maintained the depth of water throughout each trial.

The following six species of anemone were used (the abbreviations are used in the tables):

Sagartia troglodytes (Price) :	STRO
Metridium senile (L.) :	MSEN
Urticina felina (L.) :	URFE
Urticina eques (Gosse) :	UREQ
Actinia equina (L.) Green morph :	AEQG
Actinia equina (L.) Red morph :	AEQR

As discussed in Chapter 2, all anemones used in the present experiment are known to be acceptable dietary items for A.papillosa, with the exception of U.eques - an offshore sublittoral species. U.eques was included in the experiments solely because there was no possibility of these intertidal nudibranchs having previously encountered this item. This also applies to M.senile, which is often cited as the most preferred species (see above). It is possible that the nudibranchs could have encountered the intertidal species A.equina and U.felina although this is considered unlikely. With regard to A.equina it had been previously noted (pers.obs.) that A.papillosa displayed differential responses to the two colour morphs and hence these are treated as separate species.

Undamaged anemones were used in all trials and in most - but not all - cases anemones were expanded during the trial. Sizes of anemones were standardized by weight within each trial, but some between-trial differences were unavoidable due largely to problems in obtaining sufficiently small U.eques.

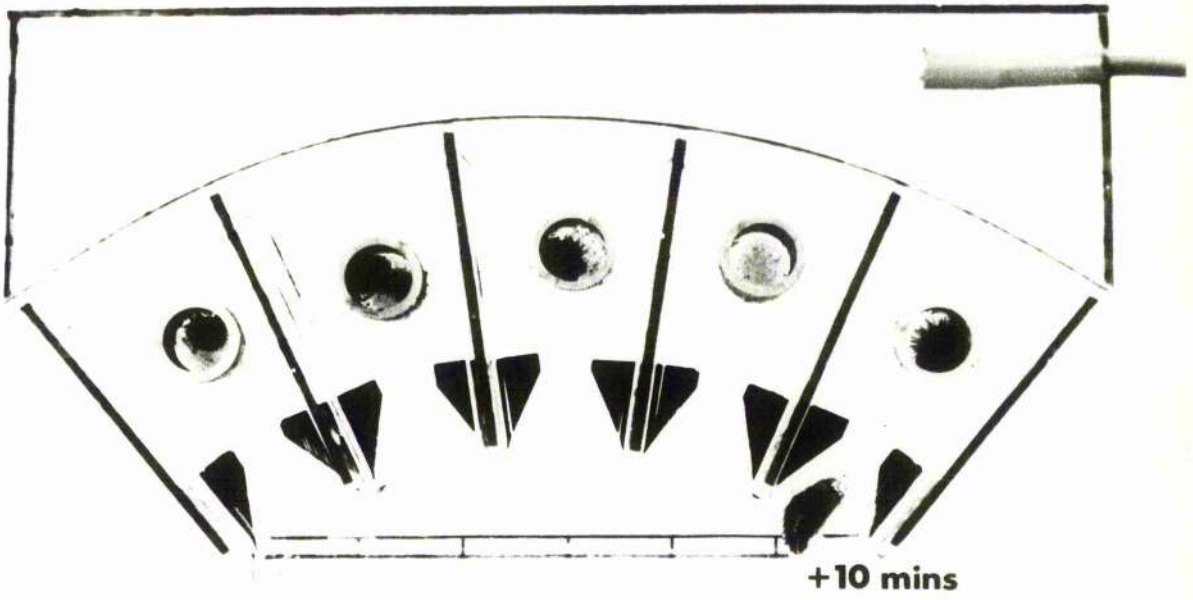
Individual aeolids were offered combinations of four of the six anemone species in each trial, leaving one 'blank' chamber which held an empty plastic cage. This ensured that on some occasions a selection would have to be made from four items which did not include the maintenance diet. In every trial (one day^{-1} nudibranch^{-1}) individuals were offered one of the 15 possible selections of four out of the six anemone species used. On any

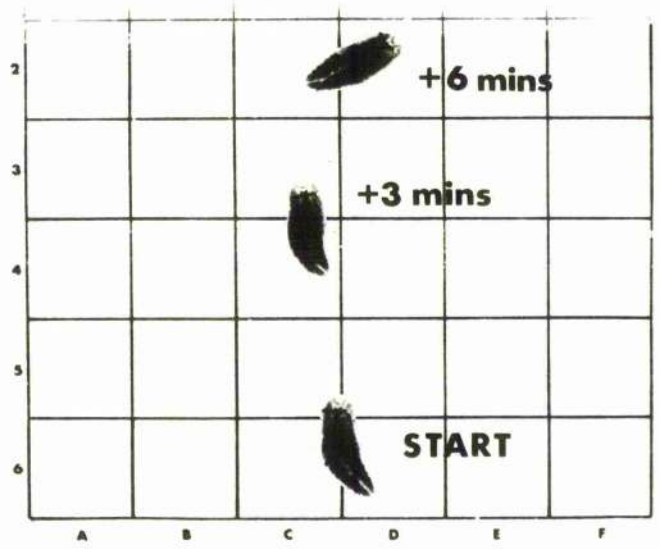
one day all 15 selections were offered, each one to a different animal, so that over the 15-day period each nudibranch would receive all combinations. This attempt to 'balance' the experiment was aimed at reducing any bias in the results through behavioural deviations on any particular day. The allocation of particular anemones to individual chambers was carried out so as to ensure a similar balance in the number of times each species of anemone occupied each chamber; this precludes any 'chamber effect' on the behavioural experimental results.

Each trial was commenced by placing the aeolid on the glass base plate at the point at which the five outflows converged (Plate 2, "Start"). Movements of the nudibranch were recorded using the grid on the base-plate and particular note was taken of the animal entering one or more ante-chambers before finally making a choice. The trial was completed once the nudibranch had passed through an ante-chamber, and into the main chamber holding the anemone (or blank). The total elapsed time and water temperature were noted for each trial. Time to make a choice ranged from 3 to 38 minutes. In some instances the nudibranch would crawl off the base-plate or by-pass the chamber section completely. Such animals were 're-started' but if this behaviour continued the trial was abandoned and was repeated for that individual on a subsequent date. After each trial the chamber apparatus was washed thoroughly with 'Decon 90' and rinsed before re-use.

Plate 2.

Time lapse photograph of a trial showing the typical behavioural responses of a nudibranch.


+10 mins


+6 mins
+3 mins
START
2
3
4
5
6
A
B
C
D
E
F

For the initial hierarchy the aeolids were maintained in their individual cages and fed S.troglodytes. Individuals were fed immediately after each trial but starved overnight in preparation for trial the following morning. Analysis of the initial hierarchy indicated that the red morph of A.equina was low down in preference. Since this species is frequently reported as preferred in other studies it was decided to change the maintenance diet (that is, 'conditioning' diet for the second hierarchy) to this species. The same behavioural experimental nudibranchs were maintained on red A.equina for one week following completion of the first hierarchy, at which time trials to determine the second hierarchy were commenced. The nudibranchs were maintained on red A.equina throughout the second hierarchy. In order to ascribe statistical significance to the prey-preference hierarchies a statistical model has been derived.

This model utilizes a branch of statistics known as likelihood theory - an area of estimation theory that is not commonly used in biological research. In view of this, an introduction to likelihood theory is provided before the statistical model is described. The development of the model itself and my own introduction to this branch of statistics is entirely attributable to Dr A.D.Gordon, Dept of Statistics, University of St Andrews. The description of the model presented below has been provided, almost entirely, by Dr Gordon although the introduction to likelihood theory is largely my own albeit

drawn heavily from reference texts (especially Mood et al, 1974, Chapter VII).

AN INTRODUCTION TO LIKELIHOOD THEORY

Simply stated, likelihood theory provides a means by which a 'best estimate' of an unknown population parameter is obtained from a set of observations from that population.

Let X denote a random variable which represents a characteristic of the elements of a population. The <u>form</u> of the density function of X ($f(.;\theta)$) is assumed known except that it contains an unknown parameter θ. Furthermore, assume that the values $x_1, x_2, \ldots, x_n$ of a random sample $X_1, X_2, \ldots, X_n$ from the density function ($f(.;\theta)$) can be observed. The objective is to estimate the value of the unknown parameter θ, or the value of some function of the parameter (e.g. $\tau(\theta)$), using the observed sample values $x_1, x_2, \ldots, x_n$.

One way in which this may be achieved is by <u>point estimation</u> where the value of some statistic, say $t(X_1, \ldots, X_n)$, is allowed to represent, or estimate, the unknown (θ); such a statistic is known as a <u>point estimator</u>. For example, the value $\bar{x}$ of the statistic $\bar{X}$, computed from a sample of size n, is a point estimate of the unknown population parameter μ - the true value of the mean for some population characteristic. In this case the point estimator is the statistic $\bar{X}$.

The method of maximum likelihood is, perhaps, the most important means by which the 'best' estimate of θ is chosen from all the possible estimators. In our notation let $\hat{\theta}$, denote an estimate of the fixed but unknown parameter θ.

In order to proceed, it is necessary to introduce the likelihood function. Let $X_1,\ldots,X_n$ denote a random sample from the density function $f(x;\theta)$. The likelihood function is defined to be the joint density function of the n random variables, in this case, $f(x_1;\theta)\ f(x_2;\theta)\ \ldots\ldots f(x_n;\theta)$; the usual notation for this is $L(\theta;x_1,\ldots,x_n)$. The likelihood function gives the likelihood of θ given that the random variables assume a particular set of values $x_1,x_2,\ldots,x_n$.

Suppose that the joint density of n random variables is $fx_1,\ldots,x_n\ (x_1,\ldots,x_n;\theta)$, where θ is unknown, and let the particular values which are observed be represented by $x_1',x_2',\ldots,x_n'$. We want to know from which density function (what value of θ) is the likelihood greatest that the set $x_1',x_2',\ldots,x_n'$ was obtained. In other words, we want to find the value of θ, denoted by $\hat{\theta}$, which maximises the likelihood function $L(\theta,x_1',\ldots,x_n')$. The maximum value of θ obtained is termed the maximum likelihood estimate. This may be obtained numerically, by trying many possible values of θ, and choosing the one which maximises the likelihood function.

The basic principles of maximum likelihood estimation, outlined above, may be illustrated with the following simple example:

Consider a simple random experiment in which a coin is tossed twice. Assume that p, the probability of obtaining a head, is unknown. We want to make inferences about p from the n=2 observations that we have obtained.

The probability that r heads will be observed out of n tosses of the coin is proportional to:

$$p^r (1-p)^{n-r}$$

The above function is the likelihood function since it gives the probability that the random variable R (the number of heads) takes the value r; for discreet random variables the likelihood is a probability.

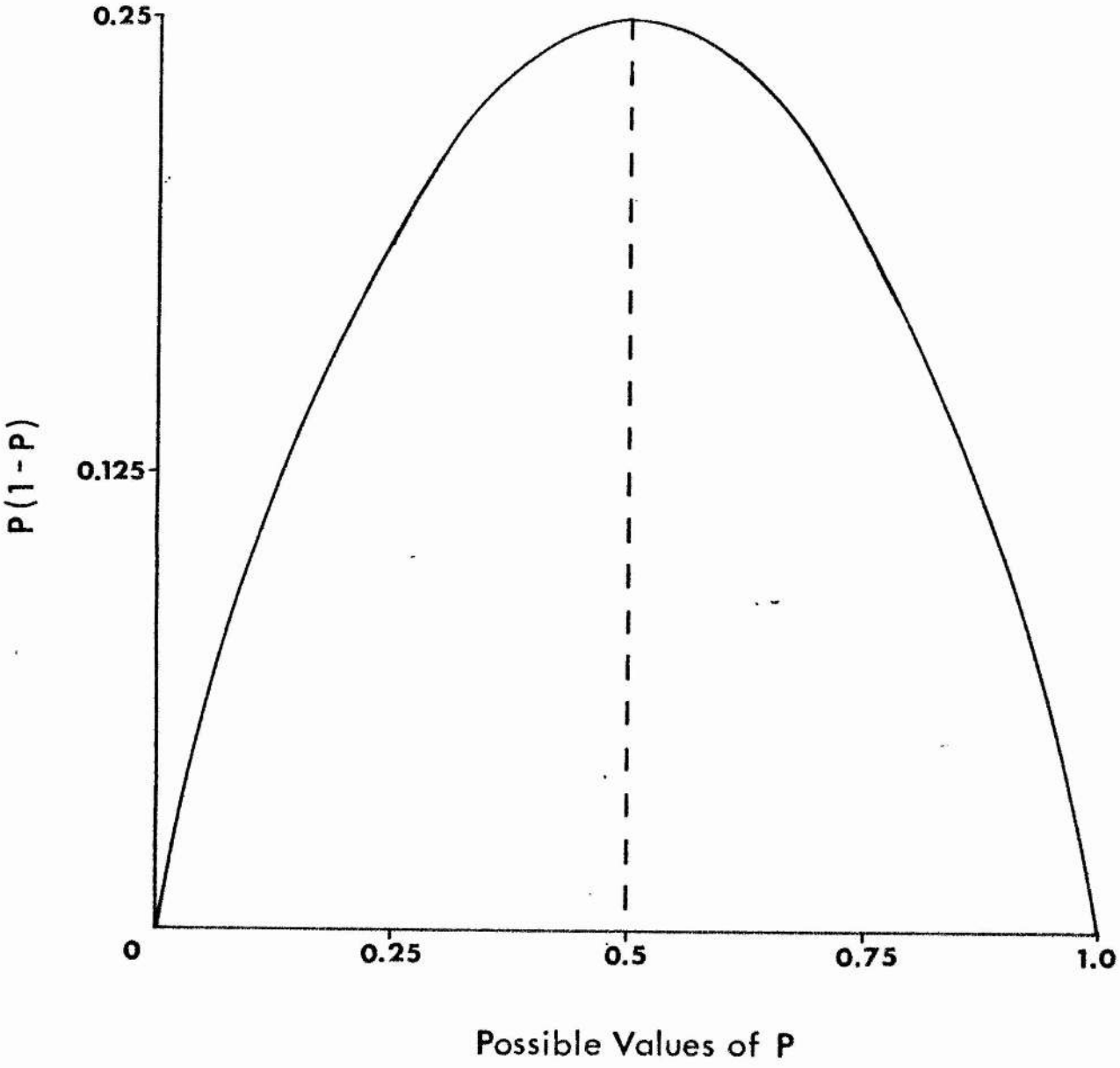
Consider tossing a coin twice and observing one head (n=2, r=1). The likelihood that this result would be obtained for any given value of p is:

$$\alpha \; p^1(1-p)^{2-1} \quad \text{or} \quad p(1-p).$$

Choosing values for p we can obtain values for this likelihood, and we choose, as our best estimate, the value of p which

Figure 7.

Graph showing the maximisation of the likelihood function $p(1-p)$ to give a maximum likelihood estimate of 0.5 for P. (For explanation see text).


0.25
0.125
P(1-P)
0
0.25
0.5
0.75
1.0
Possible Values of P

maximises the function $p(1-p)$. The maximum likelihood estimate in this case is 0.5 and is shown in figure 7. Using the previous notation, p is the unknown parameter, and r is an observation of the random variable R (the number of heads); formerly denoted by x and X respectively.

STATISTICAL MODEL FOR BEHAVIOURAL EXPERIMENT 1

This model was entirely derived by Dr A.D.Gordon. Most of the description which follows is attributable to Dr Gordon and I gratefully acknowledge his contribution to this work.

In the experimental design, of the six prey species under consideration, only four were on offer at any one time: the predator can only indicate a preference for one of these.

Let P_{im} denote the probability that diet i will be preferred to the group of diets m; m comprises the remaining three species in any one trial. The model assumes that P_{im} is constant through the experiment (over both time and separate individuals) and that the trials are independent of one another. It is possible, however, that trials may not be completely independent of one another since the prey choice made in one trial may affect subsequent choices. While such dependence is possible, it is not considered likely owing to the length of time between trials and the absence of choice reinforcement through feeding.

Let r_{im} denote the number of times that diet i was selected when i+m were on offer. If the set of probabilities (P_{im}) were known, then the probability of observing a set of outcomes (r_{im}) is proportional to the product of terms like:

$$P_{im}^{r_{im}} \qquad (1)$$

where the product is taken over (i) the group of four species on offer and (ii) all 15 ways of selecting any four from the six anemone species. In the present experiment, the set (P_{im}) is not known, but we observe (r_{im}) and can, therefore, make inferences about (P_{im}).

Given the set of experimental results (r_{im}), the likelihood of values (P_{im}) is proportional to the product of the terms:

$$P_{im}^{r_{im}}$$

This model has 15 x 3 unknown parameters - there are 15 ways of selecting four from six anemones and three unknown probabilities for the selection of any four anemone species (because we can derive the fourth probability as one, minus the sum of the other three)

In order to make progress, we need to formulate a more parsimonious model for the set (P_{im}). One standard method is to assume that each species has a certain 'merit' or 'attractiveness'.

If the attractiveness of the ith species is π_i, and π_m denotes the sum of the π-values in the group m, the probability that species i will be selected when i+m are on offer is given by

$$P_{im} = \frac{\pi_i}{\pi_i + \pi_m} \qquad (2)$$

An indeterminacy in this equation, due to the fact that multiplying each π-value by the same constant would leave P_{im} unchanged, can be removed by requiring that the sum of the π_i is unity. In this model, the number of unknown parameters has been reduced from forty-five to five.

The likelihood (L) of the unknown π_i is proportional to the product (over the same sixty terms as in expression 1) of:

$$\left(\frac{\pi_i}{\pi_i + \pi_m}\right)^{r_{im}}$$

What we seek are the values of $(\pi_1, \ldots, \pi_6)$ which maximise the likelihood $L(\pi_1, \ldots, \pi_6)$ (subject to $\sum_{i=1}^{6} \pi_i = 1$): i.e. we seek those π-values which are most consonant with the experimental data. These values are termed the maximum likelihood estimates, denoted by $\hat{\pi}_i$; they can be obtained numerically (along with

their associated standard errors) with the assistance of an iterative function-maximization computer program. For a general account of maximum likelihood estimation, see Mood et al. (1974, Chapter VII).

If there are no differences in attractiveness between the six prey species, each $\prod_i = \frac{1}{6}$. We can assess how acceptable this is as a hypothesis by comparing the value taken by the likelihood under this null hypothesis with the maximum value it attains when the $\prod$ i's are derived from the experimental results. This likelihood ratio test is effected by comparing

$$2 \log[L(\hat{\prod}_1, \hat{\prod}_2, \hat{\prod}_3, \hat{\prod}_4, \hat{\prod}_5, \hat{\prod}_6)/L(\tfrac{1}{6}, \tfrac{1}{6}, \tfrac{1}{6}, \tfrac{1}{6}, \tfrac{1}{6}, \tfrac{1}{6})]$$

with a χ^2_5 distribution (Mood et al. 1974, Chapter IX). With this test, the hypothesis that the $\prod$ i-values are equal is in fact, convincingly rejected for both sets of trials. A more general account of the principle of likelihood ratio tests is given later in this chapter.

The test outlined above provides no information about the nature of the indicated differences between the attractiveness of prey species; these can be explored with the assistance of the set of fifteen variables Z_{ij} defined by

$$Z_{ij} = \frac{\left(\hat{\prod}_i - \hat{\prod}_j\right)}{\text{S.E.}\left(\hat{\prod}_i - \hat{\prod}_j\right)}, \qquad (3)$$

where S.E. $(\hat{\Pi}_i - \hat{\Pi}_j)$ denotes the standard error of $(\hat{\Pi}_i - \hat{\Pi}_j)$. If $\Pi_i = \Pi_j$, Z_{ij} is approximately a standard Gaussian variable, with zero mean and unit variance; hence the magnitude of Z_{ij} gives an indication of how likely it is that $\Pi_i = \Pi_j$. For a single pre-specified Z_{ij}, a value which is greater than 1.96 indicates that it is unlikely (probability $P < 0.05$) that $\Pi_i = \Pi_j$.

There are dangers, however, in (i) merely comparing each of the fifteen Z-values with a standard Gaussian distribution and quoting the associated significance level; and (ii) in allowing the results of the experiment to dictate the comparisons on which one concentrates attention. Furthermore, we wish to make a composite statement about the relationships between the attractiveness of all the species which has a high probability of being correct in all its assertions about differences in attractiveness.

The problem of multiple comparison tests and the choice of a significance level for each pairwise comparison is discussed in Hall et al. (1982) and will not be considered here; suffice it it to say that the maintenance diet is regarded as preferred to another species if the corresponding Z-value exceeds 2.08; all comparisons in which the maintenance diet is not included utilize a Z-value of 2.33. This is because we are able to consider tests regarding the maintenance diet as one-sided, since we could reasonably specify the hypothesis that the maintenance diet would

be preferred before the start of the experiment.

RESULTS.

Tables 16 and 17 summarize the results of trials for molluscs maintained on S.troglodytes. Table 16 shows the number of times each mollusc selected each species of anemone during the course of the experiment. In a balanced experiment, each mollusc would make 15 choices, and a total of 270 decisions would be recorded for a collection of 18 molluscs. Further, if no trial had to be repeated, each species of anemone would be offered 10 times to each mollusc. As can be seen from Table 16 the experiment is not completely balanced; the following three factors account for this feature of the data.

(i) Mollusc 6 died during the period of the experiment; its set of trials was completed by mollusc 19.

(ii) On two occasions (i.e. for molluscs 3 and 18), the same set of anemones was inadvertently offered more than once to a mollusc during its set of trials.

(iii) More than 20% (73 out of 332) of the trials were terminated when the mollusc had not selected a chamber after 30 minutes. Often, a repetition of the trial failed to produce a decision. Obtaining a completed set of trials was expected to require a disproportionate amount of time relative to the information obtainable; accordingly, the experiment was terminated at the stage recorded in Table 16.

Table 16. Results of trials undergone by molluscs maintained on Sagartia troglodytes.

		MOLLUSC																			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	Total
Chamber entered	AEQR	1	1	2	1	0	2*	1	1	1	2	1	0	3	2	2	1	1	4*	1	27
	AEQG	2	1	4*	3	1	0	2	2	1	1	3	3*	3	3	1	3	1	3	1	38
	UREQ	1	0	3	3	2	1	2	4*	4*	2	3	2	0	2	2	5*	4	0	1	41
	URFE	1	1	0	0	0	1	0	1	1	0	2	1	1	0	1	0	0	1	0	11
	MSEN	6*	4	4	0	3	0	1	2	1	2	2	3	2	2	4*	1	1	2	3*	43
	STRO	2	2	1	3	6*	1	4	4	4	6*	4*	4*	5*	3	4*	3	6*	2	4*	68
	BLANK	2	5	2	5	2	0	2	0	2	2	0	2	1	3	1	0	0	2	0	31
Number of completed trials		15	14	16	15	14	5	12	14	14	15	15	15	15	15	15	13	13	14	10	259
Number of terminated trials		3	6	3	4	7	5	6	5	5	3	3	0	4	1	1	7	7	2	1	73

* Denotes trials in which an anemone was selected at least one-third of the times it was offered.

Table 17. The total number of times each set of four species of anemone was offered and a selection made, by molluscs maintained on Sagartia troglodytes. The two species not present in the set of four are those specified by the row and column in which the number appears.

AEQG	15				
STRO	14	17			
MSEN	16	15	14		
UREQ	16	15	14	15	
URFE	16	16	16	15	14
	AEQR	AEQG	STRO	MSEN	UREQ

Instances in which a particular species was selected by a mollusc at least one third of the times it was offered are denoted by an asterisk in Table 16. The completion rate of trials was consistently high for some individuals (e.g. No. 14) in contrast to others (e.g. No. 5), which made a choice much less often. In general, however, individual variability is not great. This is reflected by the consistency of extremes of response to, for example, S.troglodytes and U.felina. A likelihood ratio test of the hypothesis that all of the molluscs had the same set of $\prod i$'s could not be rejected. In this context it is, perhaps, surprising that U.felina should be selected so infrequently (11 times) in contrast to its congener U.eques (41 times), the latter being exclusively sublittoral and offshore in distribution. The hierarchy for the summed performances of all the nudibranchs does, however, reflect a distinct polarity of response with S.troglodytes (the field and maintenance diet) the most preferred and U.felina the least preferred item.

Table 18 presents the Z-values derived from the statistical model and from these we can differentiate the significance of specific differences within the prey-preference hierarchy so derived. From the table it is apparent that S.troglodytes is preferred over all other anemone species and that, of the remaining five species, we can significantly differentiate a lack of preference for U.felina. That is to say that, in terms of preference, S.troglodytes > M.senile = U.eques = A.equina green

Table 18. The set of Z-values resulting from the application of the statistical model to the data obtained from molluscs maintained on Sagartia troglodytes. The row and column in which a number appears specify the pair of species which are being compared.

AEQR	2.642*				
AEQG	4.000*	1.472			
UREQ	4.245*	1.704	0.216		
MSEN	4.470*	1.955	0.471	0.258	
STRO	6.954*	4.548*	3.100*	2.898*	2.638*
	URFE	AEQR	AEQG	UREQ	MSEN

* Denotes Z-value greater than 2.63

= A.equina red > U.felina.

On the basis of this result, and in view of the frequent citation of A.equina in previous preference studies, the maintenance diet was changed to the red morph of this species. The nudibranchs were so maintained for one week before recommencing trials and were similarly fed on red Actinia throughout the trial period.

Tables 19 and 20 summarize the results of trials for molluscs maintained on red A.equina; the format of these tables is the same as that for Tables 16 and 17. Fewer molluscs participated in the second set of trials because there was a shortage of molluscs at this stage; some individuals were replaced by animals already participating in the experiment.

It is noteworthy that in this series two prey species (red A.equina and S.troglodytes) were never totally ignored and that most nudibranchs selected red A.equina on at least a third of the occasions this prey was offered. It is apparent also that red A.equina has been elevated to the top of the hierarchy, with the initially most preferred item (S.troglodytes) relegated to third position. From the Z-values for the second hierarchy (Table 21) a somewhat different array of preferences emerges. Red A.equina is now significantly preferred over all other items, with green A.equina and S.troglodytes indistinguishable from one another, but themselves significantly more preferred than the trio of M.senile, U.eques and U.felina. That is, red A.equina > green

Table 19. Results of trials undergone by molluscs maintained on red Actinia equina.

		MOLLUSC																	
		1	2	3	5	7	8	9	10	11	12	13	14	15	17	18	19	20	Total
	AEQR	3	5*	3	1*	5*	6*	4*	3*	5*	6*	4	5*	4*	1	5*	3	3	66
	AEQG	3	3	3	3*	0	3	5*	2	3	2	3	1	3	4*	1	2	3	44
Chamber	UREQ	1	1	1	0	1	1	2	2	2	0	3	1	4	0	1	0	5	25
entered	URFE	0	2	0	0	0	1	0	2	4	1	2	1	2	5*	0	3	0	23
	MSEN	3	2	2	1	4	2	1	1	3	0	0	2	2	2	3	0	0	28
	STRO	2	5*	4	1	2	2	2	1	3	1	4	5*	1	2	2	4	2	43
	BLANK	4	2	3	0	2	0	1	1	0	0	0	2	0	1	2	3	4	25
Number of completed trials		16	20	16	6	14	15	15	12	20	10	16	17	16	15	14	15	17	254
Number of terminated trials		10	0	8	0	5	6	4	1	0	5	7	2	3	3	5	7	6	72

* Denotes trials in which an anemone was selected at least one-third of the times it was offered.

Table 20. The total number of times each set of four species of anemone was offered and a selection made, by molluscs maintained on red Actinia equina. The two species not present in the set of four are those specified by the row and column in which the number appears.

AEQG	15				
STRO	16	15			
MSEN	17	17	16		
UREQ	14	13	15	17	
URFE	15	14	15	15	15
	AEQR	AEQG	STRO	MSEN	UREQ

Table 21. The set of Z-values resulting from the application of the statistical model to the data obtained from molluscs maintained on red Actinia equina. The row and column in which a number appears specify the pair of species which are being compared.

UREQ	0.288				
MSEN	0.897	0.616			
STRO	2.644*	2.370*	1.737		
AEQG	2.677*	2.399*	1.765	0.013	
AEQR	5.044*	4.777*	4.144*	2.445*	2.445*
	URFE	UREQ	MSEN	STRO	AEQG

* Denotes a Z-value greater than 2.37.

A.equina = S.troglodytes > M.senile = U.felina = U.eques. However, the difference between M.senile and the pair (green A.equina and S.troglodytes) is indicated less strongly, and would best be regarded as a suggestion for future investigation. It is perhaps relevant that changing the maintenance diet to red A.equina did not incur any marked change in the preference for green A.equina, thus justifying their distinction. In addition U.felina has remained the least selected item. In the two sub-sets of trials, therefore, the aeolids responded similarly in selecting the maintenance diet most frequently and by maintaining broadly similar responses to the remaining four prey items offered. This one can conclude to be good evidence for ingestive conditioning of Aeolida papillosa to its prey anemones.

Table 22 shows the proportional duration of trials on which each of the six items were on offer and selected. For almost all prey species in both sub-sets of trials the selection response was similar, with most trials complete within nine minutes, but a few requiring up to thirty minutes. Perhaps the only major exception was the response to U.eques which showed an even proportionality of time responses. It is relevant to note, however, that the conditioning prey species did not elicit a more rapid response time as might have been expected.

Table 22. The proportional duration of trials on which each of the six anemone species were on offer and were selected

Duration (minutes)	Species Selected UREQ	URFE	STRO	MSEN	AEQG	AEQR	
0-9	0.62	0.64	0.60	0.48	0.58	0.52	
10-19	0.34	0.18	0.30	0.33	0.37	0.33	STRO maintained
20-29	0.02	0.00	0.09	0.12	0.05	0.15	
29+	0.02	0.00	0.01	0.07	0.00	0.00	
0-9	0.29	0.51	0.58	0.38	0.49	0.52	
10-19	0.33	0.34	0.32	0.44	0.31	0.25	AEQR maintained
20-29	0.29	0.13	0.05	0.15	0.13	0.12	
29+	0.09	0.02	0.05	0.03	0.07	0.06	

BEHAVIOURAL EXPERIMENT 2

A modified version of behavioural experiment 1 was undertaken during July and August 1982 at the Friday Harbor Laboratories, University of Washington, U.S.A. The objective of this experiment was to establish whether the ingestive conditioning phenomenon extended to N.W.Pacific A.papillosa - with its corresponding prey-species - and to investigate the effects of two changes in maintenance diet.

MATERIALS AND METHODS.

Experiments were conducted in the laboratory with ten nudibranchs collected, by diving, from the town docks at Friday Harbor. The nudibranchs were probably associated with M.senile, although they may also have been feeding upon Urticina crassicornis (Müller) and E.prolifera.

The following four anemone species were used in prey-preference trials. The abbreviations are used in the tables and figures:

Anthopleura elegantissima (Brandt)	AELE
Metridium senile (L.)	MSEN
Urticina lofotensis (Danielson)	ULOF
Epiactis prolifera (Verrill)	EPRO

Four anemones were used (compared to six in behavioural experiment 1), because this reduced the number of trials required to confidently determine preferences in the upper part of the hierarchy. Inevitably, this resulted in less resolution of the

lower part of the hierarchy since the most preferred item was present in every trial. The number of trials required to confidently resolve a complete preference hierarchy was considered to be prohibitively large, and attention was mainly focused, therefore, on the two most preferred species.

The nudibranchs were divided into two equal groups (A and B) Each group was maintained on a given anemone diet for ten days and, in the latter five days, prey-preference trials were performed. The maintenance diet was then changed and the nudibranchs were allowed to feed uninterrupted for a further five days; prey-preference trials were then repeated in the following five days. This was repeated once more to provide prey-preference data for three different maintenance diets. Nudibranchs in Group A were maintained on A.elegantissima for the first set of trials, E.prolifera for the second set, and M.senile for the final set. Group B was treated in an identical manner except that the order in which the maintenance diets were presented was reversed (i.e. M.senile → E.prolifera → A.elegantissima). The experiment was run in opposite directions for two reasons. First, to limit the effect that the death of nudibranchs (or a reduction in their responsiveness over the experimental period) would have on the size of the data set for the final anemone diet, and secondly, to investigate the effects of direction of diet change on prey-selection responses.

Allocation of anemone species to chambers for each trial was undertaken in a similar manner to behavioural experiment 1 such that each anemone occupied each chamber an equal number of times. The additional complication of selecting the anemones for presentation in each trial was removed, however, since all four species were presented on every occasion. With the removal of this complication it became less important to repeat terminated trials since they could not bias the results through imbalances in frequency of presentation of anemone species. The relative positions of prey anemones to one another was also balanced to remove any bias that may have resulted from two species consistently occurring next to one another.

For each day upon which trials were run, every nudibranch was required to make a choice on eight separate occasions. In behavioural experiment 1 each nudibranch was required to make one choice per day in order to reduce any effects of diurnal behavioural variations; such 'balancing' was not possible in this experiment owing to the limited time available. The results of this experiment did not suggest that such behavioural deviations are important. In every other respect the methodology employed was the same as for behavioural experiment 1.

Since the objective of this experiment was to investigate the effects of ingestive conditioning and dietary history on prey-species selection, the statistical model used in behavioural experiment 1 is inappropriate. In order to ascribe statistical

significance to these data, therefore, a second statistical model has been derived. This is again attributable to Dr A.D.Gordon although much of the description was written by myself.

STATISTICAL MODEL FOR EXPERIMENT 2

Briefly stated, this analysis uses likelihood ratio tests to investigate the adequacy of a series of increasingly restricted models to describe the observed nudibranch responses. In the first part of this section the models themselves will be described; this will be followed by an outline of the principles of likelihood ratio tests.

In the statistical model described earlier for behavioural experiment 1 it was necessary to formulate a more parsimonious model for the set P_{im}. This was achieved by assuming that each anemone species had a certain 'merit' or 'attractiveness'. Under this assumption a more restricted model was formulated which had only five unknown parameters. Similarly, we are able to construct a set of increasingly restricted models (i.e. with fewer unknown parameters) for the nudibranch responses we observe in the present experiment.

The models are constructed as follows and are presented in decreasing order of restriction.

Because each diet is on offer in every trial, the responses of molluscs can be modelled by a multinomial distribution. In what follows the blank chamber selections are ignored.

Let P_{ijkl} denote the probability that mollusc i, conditioned on anemone k, in experiment j (i.e. A or B), selects anemone l (i=1,....,5; k=1,....,3; j=1,2; l=1,....,4).

Model M_0: $P_{ijkl}=P_j$

In this model, the hypothesis is that all molluscs have the same pattern of response irrespective of conditioning diet (values of k) or past history of diet (values of j). This model has three unknown parameters since if we specify the probability of selecting three of the anemone diets, the fourth is known (as they sum to one).

Model M_1: $P_{ijkl}=P_{kl}$

In this model, all molluscs conditioned on the same diet have the same pattern of response, irrespective of past dietary experience. This model has 3 x 3 = 9 parameters.

Model M_2: $P_{ijkl}=P_{jkl}$

In this model, all molluscs with the same dietary history have the same pattern of response. This model has 2 x 3 x 3 = 18 parameters.

Model M_3: $P_{ijkl}=P_{ijkl}$

This is the most general model in the series and can be considered as a model of each different mollusc's individual pattern of response, for example, molluscs with the same dietary history can have different patterns of response. If a completed data set was available this model would have 90 parameters - the total number of values for the number of selections of each anemone by each mollusc on every conditioning diet i.e. 5 x 2 x 3 x 3. In this experiment, however, some data are missing, resulting in only 66 parameters.

Let the subscript q represent any of the subscripts used in the models outlined above (i.e. 1, kl, jkl or ijkl). In a manner similar to the model for behavioural experiment 1 the likelihood of the values P_q is proportional to the product of the terms

$$P_q^{r_q},$$

where r_q are the observed results for a given model. If n_q denotes the total of all values for r_q, for a given model, then the maximum likelihood of each model is proportional to the product of the terms

$$(r_q/n_q)^{r_q}.$$

The log max-likelihood values for each model are, therefore, equal to

$$c + \sum_q [r_q \log r_q - r_q \log n_q].$$

From the derived log max-likelihood values we are able to compare increasingly more restricted models (i.e. those with fewer parameters) with the most general model (M_3) for data with no specifically predicted structure. Consider a more restricted model, for example M_2. The value of the maximum likelihood under this model must be less than or equal to the maximum likelihood value for M_3, since M_3 has a larger number of parameters with which to describe the data and, therefore, produces the 'best' (i.e. largest) likelihood estimate. If, however, M_2 is a realistic model, the ratio L_{M_2}(18 parameters)/L_{M_3}(66 parameters), should be close to 1, and $-2\log[L_{M_2}(....)/L_{M_3}(....)]$ should be close to zero.

Maximum likelihood theory provides a test for examining whether M_2 can be regarded as an adequate model by comparing the term

$$-2\log\,[L_{M_2}(.,....,.)/L_{M_3}(.,....,.)],$$

with a χ^2 distribution: large values of this ratio lead to the rejection of the hypothesis that M_2 is an adequate model (see Mood et al., 1974, for a more complete account of likelihood

ratio tests). By comparing models two at a time we are able to determine whether more restricted models provide an adequate description of the data, thereby allowing us to draw conclusions regarding the responses of the nudibranchs.

RESULTS.

Tables 23 and 24 show the results of trials for groups A and B respectively; the absolute and relative distribution of prey anemone selections are presented in Figs. 8 and 9 respectively. In a completed data set, for nudibranchs conditioned to a given anemone species, each of the five nudibranchs would make a total of 40 choices. As can be seen from Tables 23 and 24 the data sets for the experiment are not complete: only 647 choices were made from a maximum possible of 1,200. The following factors account for this feature of the data.

(i) 2 nudibranchs from Group A, and 3 from Group B died during the course of the experiment; these nudibranchs could not be replaced.

(ii) on some occasions nudibranchs failed to make a decision.

Although a large proportion of the intended trials could not be completed, due to the factors outlined above, a sufficient number were completed to permit reliable statistical analysis.

Table 25 presents the log max-likelihood ratios for each of the statistical models and the results of the pair-wise comparison of more restricted models. By comparing the values in this manner the adequacy of more restricted models was evaluated.

Table 23. Results of trials undergone by Group A (AELE ►EPRO ►MSEN). in experiment 2.

		Conditioned to AELE — Mollusc						Conditioned to EPRO — Mollusc						Conditioned to MSEN — Mollusc					
		A1	A2	A3	A4	A5	TOTAL	A1	A2	A3	A4	A5	TOTAL	A1	A2	A3	A4	A5	TOTAL
Chamber Entered	AELE	6	15	10	10	7	48	1	6	5	4	5	21	-	5	7	-	0	12
	EPRO	10	13	10	7	10	50	2	16	18	20	14	70	-	14	17	-	15	46
	MSEN	11	5	6	9	3	34	2	6	12	6	7	33	-	8	6	-	8	22
	ULOF	4	1	5	1	2	13	1	4	2	2	3	12	-	4	3	-	3	10
	Blank	9	6	8	10	10	43	2	8	2	8	11	31	-	9	6	-	5	20
Trials when an anemone was chosen		31	34	31	27	22	145	6	32	37	32	29	136	-	31	33	-	26	90
Completed trials		40	40	39	37	32	188	8	40	39	40	40	167	-	40	39	-	31	110
Terminated trials		0	0	1	3	8	12	*	0	1	+	0	1	-	0	1	-	9	10

* Denotes Mollusc died
\+ Denotes Mollusc died after completing a data set

Table 24. Results of trials undergone by Group B (MSEN ► EPRO ► AELE). in experiment 2.

		Conditioned to MSEN — Mollusc						Conditioned to EPRO — Mollusc						Conditioned to AELE — Mollusc					
		B1	B2	B3	B4	B5	TOTAL	B1	B2	B3	B4	B5	TOTAL	B1	B2	B3	B4	B5	TOTAL
Chamber Entered	AELE	8	8	4	2	5	27	-	6	-	-	3	9	-	9	-	-	7	16
	EPRO	8	8	6	4	10	36	-	13	-	-	17	30	-	9	-	-	5	14
	MSEN	12	11	9	3	15	50	-	12	-	-	14	26	-	7	-	-	8	15
	ULOF	9	7	9	0	3	28	-	4	-	-	2	6	-	8	-	-	11	19
	Blank	3	5	12	1	7	28	-	5	-	-	4	9	-	7	-	-	9	16
Trials when an anemone was chosen		37	34	28	9	33	141	-	35	-	-	36	71	-	33	-	-	31	64
Completed trials		40	39	40	10	40	169	-	40	-	-	40	80	-	40	-	-	40	80
Terminated trials		+	1	+	*	0	1	-	0	-	-	0	0	-	0	-	-	0	0

* Denotes mollusc died
\+ Denotes mollusc died after completing a data set

Table 25. Log maximum likelihood values for the statitical models and the results of likelihood ratio tests for the adequacy of more restricted models as descriptions of the data from Experiment 2.

Model (M_x)	Log max-likelihood (L_x)	Likelihood Ratio $[2(L_{x+1} - L_x)]$
M_0	- 854.1422	31.99 ***
M_1	- 838.1469	34.05 ***
M_2	- 821.1239	47.0 N.S
M_3	- 797.6248	

Conclusion: Model M_2 cannot be rejected.

From the table it is apparent that for M_0 versus M_1, M_0 is convincingly rejected; for M_1 versus M_2, M_1 is convincingly rejected and for M_2 versus M_3, M_2 cannot be rejected. Thus we conclude that not only conditioning diet, but also past dietary experience influences the pattern of response.

In order to visualise the patterns of response shown under each conditioning treatment Figs. 8 and 9 present, respectively, the absolute (scaled values where less than five molluscs were used) and proportional selection of each anemone. It should, perhaps, be pointed out that the statistical analysis is performed on the absolute data and conclusions drawn from this experiment must, similarly, be based upon the absolute numerical responses of the nudibranchs. In order to facilitate comparison, however, the patterns of response can be followed in Fig. 9 which shows the proportional selection frequencies. In most cases the patterns described from the proportional data are reflected absolutely. As a result of reduced group sizes, however, the absolute data indicate a disproportionate preference for M.senile in A(iii) and M.senile and E.prolifera in B(ii).

Examination of the pattern of response to M.senile by Group B in Fig. 9 (B(i) to B(iii) reading from right to left) illustrates the conclusion regarding conditioning and past history of diet. The number of times M.senile is chosen is high while the nudibranchs are conditioned to this species and remains high following a change in maintenance diet to E.prolifera.

Figure 8.

Histograms showing the numbers of trials in which each anemone species was selected under the three conditioning regimes of behavioural experiment 2.

Asterisks denote instances where the values have been appropriately scaled in order to permit comparisons between data generated with reduced group sizes.

* Denotes three nudibranchs were used.

Scaling factor = 1.66

** Denotes two nudibranchs were used.

Scaling factor = 2.5

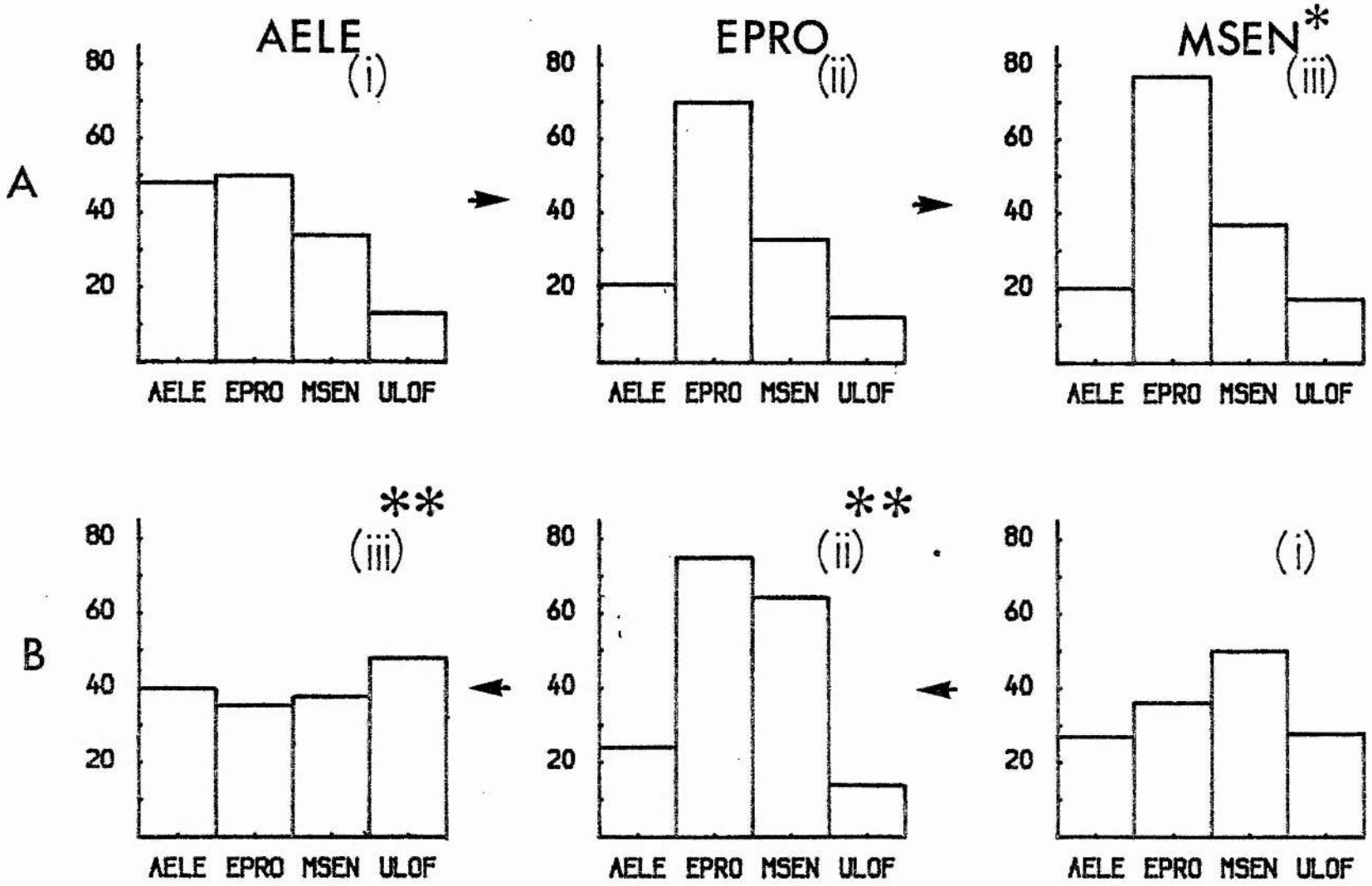
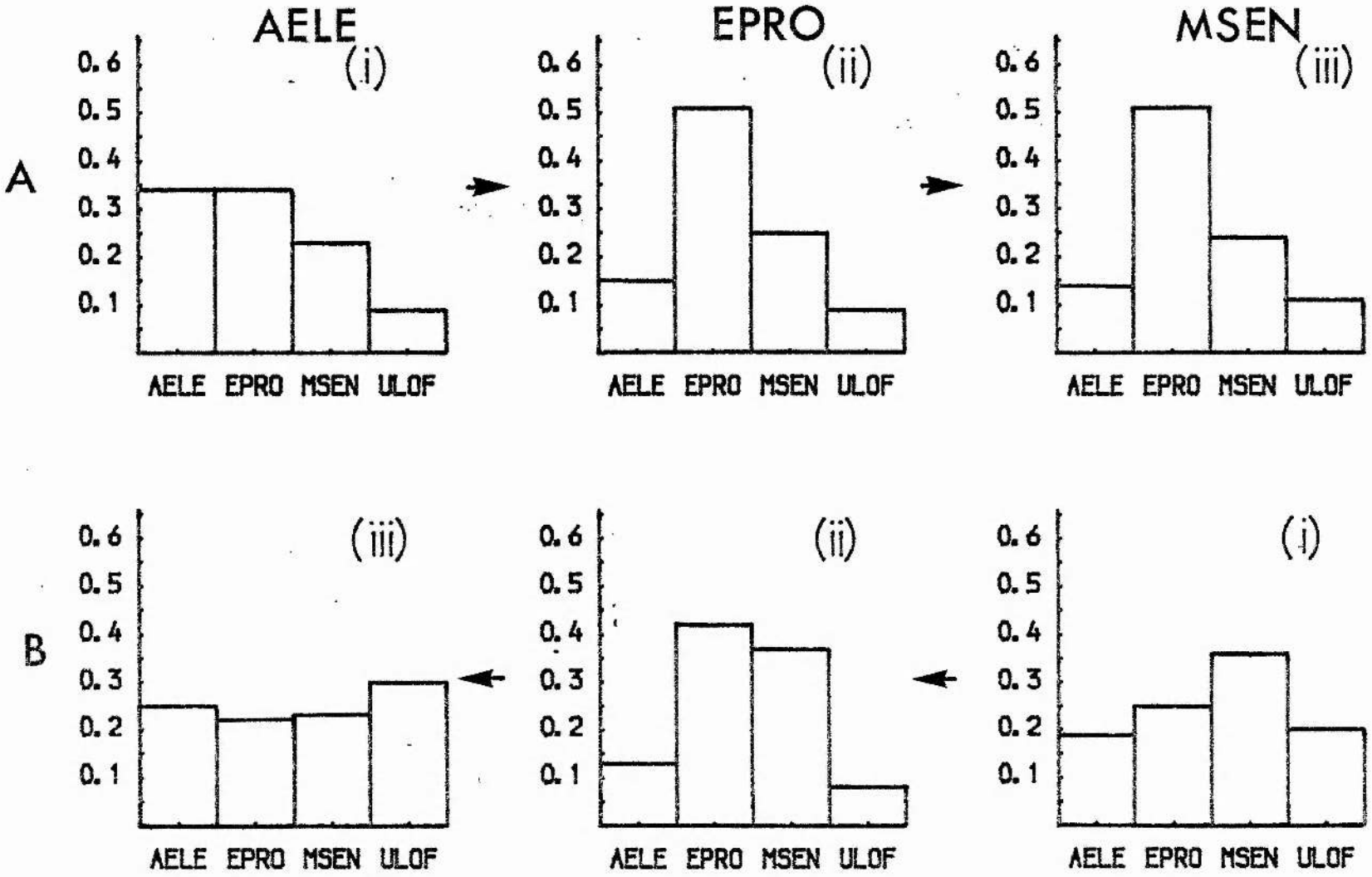

A
AELE
(i)
EPRO
(ii)
MSEN*
(iii)
80
60
40
20
AELE EPRO MSEN ULOF
B
**
(iii)
**
(ii)
(i)

Figure 9.

Histograms showing the proportion of trials in which each anemone species was selected under the three conditioning regimes of behavioural experiment 2.


A
AELE
(i)
EPRO
(ii)
MSEN
(iii)
0.6
0.5
0.4
0.3
0.2
0.1
AELE EPRO MSEN ULOF
B
(iii)
(ii)
(i)

However, the number of times E.prolifera was taken is actually greater and in the absolute data a rise in M.senile selection is predicted from the scaling. Selection of M.senile falls by the time the diet is changed to A.elegantissima (B(iii)). A similar pattern can be seen for E.prolifera in Group A (Fig. 9); the proportional selection frequency rises from A(i) to A(ii), following the change in conditioning diet to E.prolifera, and then remains high after the final change to M.senile shown in A(iii). Again, the scaling factor for the absolute values predicts a slight rise in the selection frequency of E.prolifera in A(iii) (Fig. 8).

Following a change, a continuing high selection frequency for the previous maintenance diet was not always observed. For example, the proportional selection frequency of A.elegantissima (Fig. 9) falls from A(i) to A(ii) immediately following the change in maintenance diet from A.elegantissima to E.prolifera.

Because data are more plentiful for nudibranchs conditioned to M.senile, the conclusions are somewhat biased towards differences detected in these sets of trials. The overall pattern of response is somewhat less clear than in behavioural experiment 1: this is almost certainly due to the small group sizes following the death of individuals during the experiment. Although different sets of nudibranchs were used in Groups A and B, these (and the previous) results suggest that differences are unlikely to be caused by differences in the behaviour of individual molluscs.

An examination of Fig. 9 shows that Group A's preference for E.prolifera carries over to the trials following their conditioning onto M.senile. This suggests that it may be more difficult to 'remove' conditioning to E.prolifera than it is for other species. The frequency with which E.prolifera was selected in all sets of trials suggests that this species may be a highly preferred item regardless of the complexities of ingestive conditioning.

BEHAVIOURAL EXPERIMENT 3

The objective of this experiment was to further investigate, for European A.papillosa, the importance of past dietary history which was shown to be important in prey preference determinations for N.W. Pacific A.papillosa.

MATERIALS AND METHODS.

Two separate experiments were undertaken simultaneously between January and May 1983 using a total of 20 nudibranchs collected from Robin Hood's Bay, North Yorkshire. These nudibranchs were similar to those in the first behavioural experiment, in that they had been exclusively associated with S.troglodytes in the field.

The following four anemone species were used (the abbreviations are used in the table):

Actinia equina (Red Morph)	AEQR
Sagartia troglodytes	STRO
Metridium senile	MSEN
Urticina felina	URFE

The number of anemone species was reduced from six to four for the same reason outlined in the second behavioural experiment (i.e. to reduce the number of trials required to elucidate the upper part of the preference hierarchy).

Two groups of ten nudibranchs were used in each of two simultaneous experiments, hereafter referred to as experiments 3(i) and 3(ii) respectively. Each of these experiments was essentially similar to behavioural experiment 2 in that two groups of five nudibranchs experienced two changes in maintenance diet and, using the choice-chamber apparatus, the prey-selection responses were determined for the three maintenance diets. The direction of diet changes was reversed for one group of five in each experiment as at Friday Harbor. A summary of the diet changes for molluscs in each experiment is shown in the table below.

		1st Diet	2nd Diet	3rd Diet
Expt	Group C	MSEN	URFE	AEQR
3(i)	Group D	AEQR	URFE	MSEN
Expt	Group E	AEQR	MSEN	STRO
3(ii)	Group F	STRO	MSEN	AEQR

Each group was conditioned to its respective anemone diet for ten days before beginning the experiment since time was not restricted as in Friday Harbor. Following the completion of a set of trials the maintenance diet was changed and the nudibranchs were allowed to feed for ten days before trials were recommenced. Trials for each experiment were performed on alternate days and individuals were expected to make between one and four choices on any one day; a total of fifteen trials were

run for each individual. Each data set for a given diet took approximately one month to complete since it was not possible to run trials every day.

In every other respect the methodology employed is the same as that used in behavioural experiments 1 and 2 and the results have been analysed using the statistical models outlined for behavioural experiment 2.

RESULTS.

Behavioural Experiment 3(i).

Tables 26 and 27 show the results of trials for groups C and D respectively. In a completed data set, for nudibranchs conditioned to a given anemone species, each of the five individuals in a group would make 15 choices. As can be seen from the results, the data are not complete. This can be accounted for by the following factors:

(i) one mollusc (B3) died during the course of the experiment.

(ii) on some occasions nudibranchs failed to make a decision. A total of 354 decisions were made out of a possible 450 for a completed experiment. Despite these missing data enough decisions were made to allow analysis of the results using the statistical models from behavioural experiment 2.

Table 26. Results of trials undergone by Group C in Experiment 3(i). (MSEN ►URFE ►AEQR).

		Conditioned to MSEN Mollusc						Conditioned to URFE Mollusc						Conditioned to AEQR Mollusc					
		B1	B2	B3	B4	B5	TOTAL	B1	B2	B3	B4	B5	TOTAL	B1	B2	B3	B4	B5	TOTAL
Chamber Entered	AEQR	3	1	7	3	2	16	1	2	1	1	3	8	5	8	-	5	8	26
	STRO	3	3	2	3	5	16	5	5	1	1	4	16	3	1	-	0	0	4
	URFE	1	1	1	0	4	7	3	3	0	4	2	12	4	3	-	1	3	11
	MSEN	3	5	4	2	2	16	5	2	0	2	2	11	1	1	-	2	1	5
	Blank	3	2	1	1	1	8	1	3	0	2	2	8	2	2	-	2	1	7
Trials when an anemone was chosen		10	10	14	8	13	55	14	12	2	8	11	47	13	13	-	8	12	46
Completed trials		13	12	15	9	14	63	15	15	2	10	13	55	15	15	-	10	13	53
Terminated trials		2	3	0	6	1	12	0	0	4+	5	2	11	0	0	-	5	2	7

4+ Denotes Mollusc died after completing a data set

Table 27. Results of trials undergone by Group D in Experiment 3(i). (AEQR ► URFE ► MSEN).

		Conditioned to AEQR Mollusc						Conditioned to URFE Mollusc						Conditioned to MSEN Mollusc					
		A1	A2	A3	A4	A5	TOTAL	A1	A2	A3	A4	A5	TOTAL	A1	A2	A3	A4	A5	TOTAL
Chamber Entered	AEQR	9	4	3	4	7	27	8	7	4	5	1	25	1	6	4	6	-	17
	STRO	2	2	1	3	2	10	1	1	3	4	2	11	2	0	1	2	-	5
	URFE	3	4	2	3	1	13	2	4	5	4	0	15	1	3	4	2	-	10
	MSEN	1	1	3	2	0	7	0	0	2	0	3	5	2	1	4	3	-	10
	Blank	0	3	3	1	0	7	3	3	1	2	2	11	1	5	2	2	-	10
Trials when an anemone was chosen		15	11	9	12	10	57	11	12	14	13	6	56	6	10	13	13	-	42
Completed trials		15	14	12	13	10	64	14	15	15	15	8	67	7	15	15	15	-	52
Terminated trials		0	1	3	2	5	11	1	0	0	0	7	8	+	0	0	0	-	+

Table 28. Log max-likelihood values and likelihood ratio tests for Experiment 3(i).

Model (M_x)	Log max-likelihood (L_x)	Likelihood Ratio $[2(L_{x+1} - L_x)]$
M_0	- 404.3313	18.422 ***
M_1	- 395.1203	20.249 ***
M_2	- 384.9960	75.500 N.S.
M_3	- 347.2456	

Conclusion: Model M_2 cannot be rejected.

Table 28 shows the Log Max-Likelihood Ratios and the results of the pairwise comparisons of more restricted models. From the table it is apparent that the results indicate a similar pattern of response to that shown in behavioural experiment 2 in the U.S.A. i.e. Model M_2 cannot be rejected. From this it is concluded that, once again, not only the conditioning diet but also past dietary experience influences the pattern of response. In view of the less plentiful data in behavioural experiment 3 the significance level has been reduced to p=.05.

Figs. 10 and 11 (respectively) show the absolute and proportional number of times each species was selected, when an anemone was actually chosen, for each of the treatments in experiment 3(i). The patterns of selection are discussed in the same manner as for behavioural experiment 2: the proportional selections shown in Fig. 11 are used to illustrate the patterns and, although in most cases these are reflected in the absolute values (Fig. 10), any departures from this pattern are noted. A general trend in the pattern of anemone selection can be seen in that the frequency with which a particular species is chosen remains low until molluscs are conditioned to that species. The frequency then rises to a maximum during conditioning, and falls to an intermediate level following the change to a new conditioning diet. An example of this can be seen by following the pattern of response to U.felina through C(i) to C(iii) in Fig. 11. A related pattern can be observed for the initial conditioning diet (i.e. M.senile for group C and A.equina for group D); the proportional selection frequency of both species

Figure 10.

Histograms showing the numbers of trials in which each anemone species was selected under the three conditioning regimes of behavioural experiment 3(i).

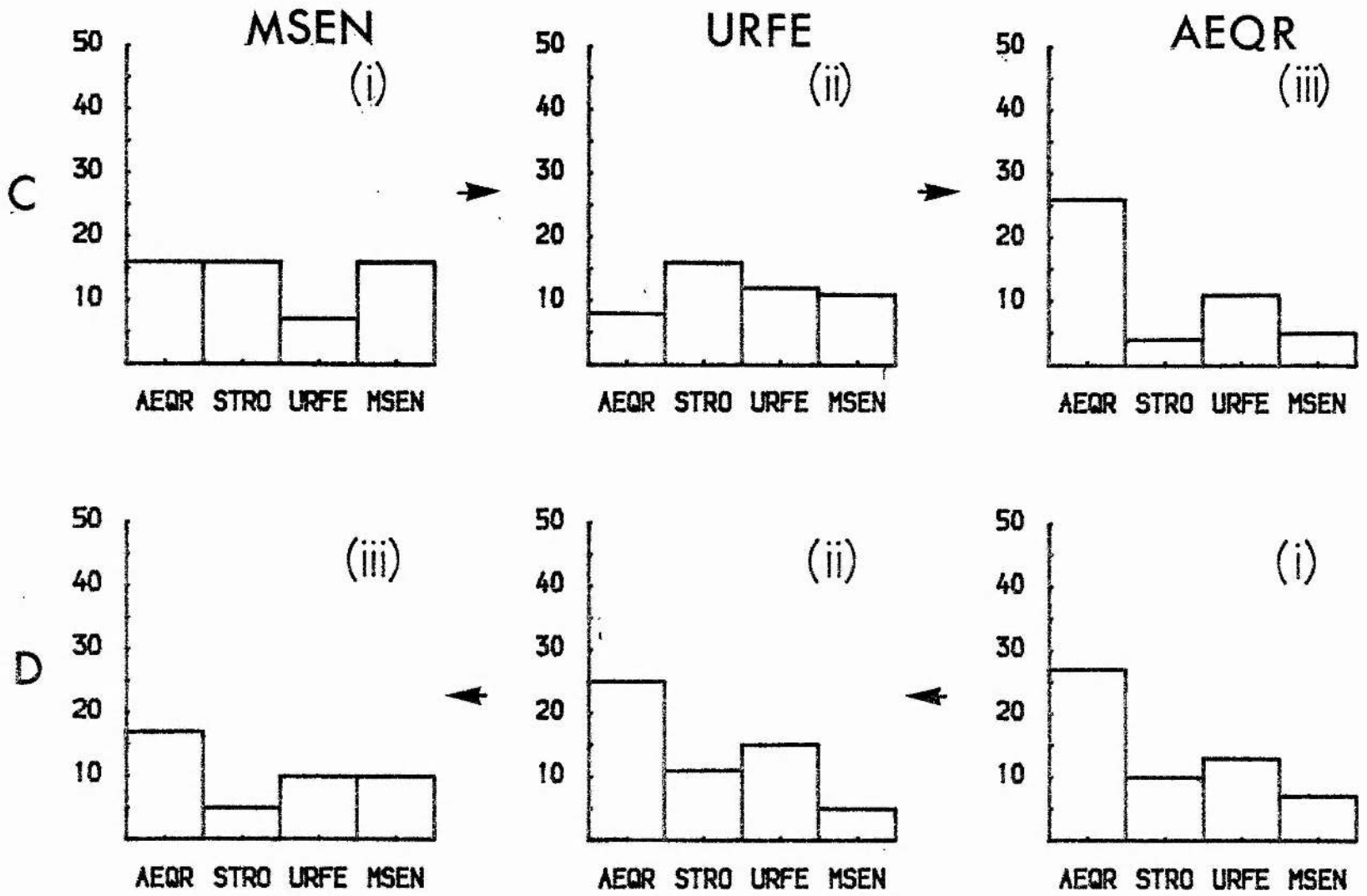
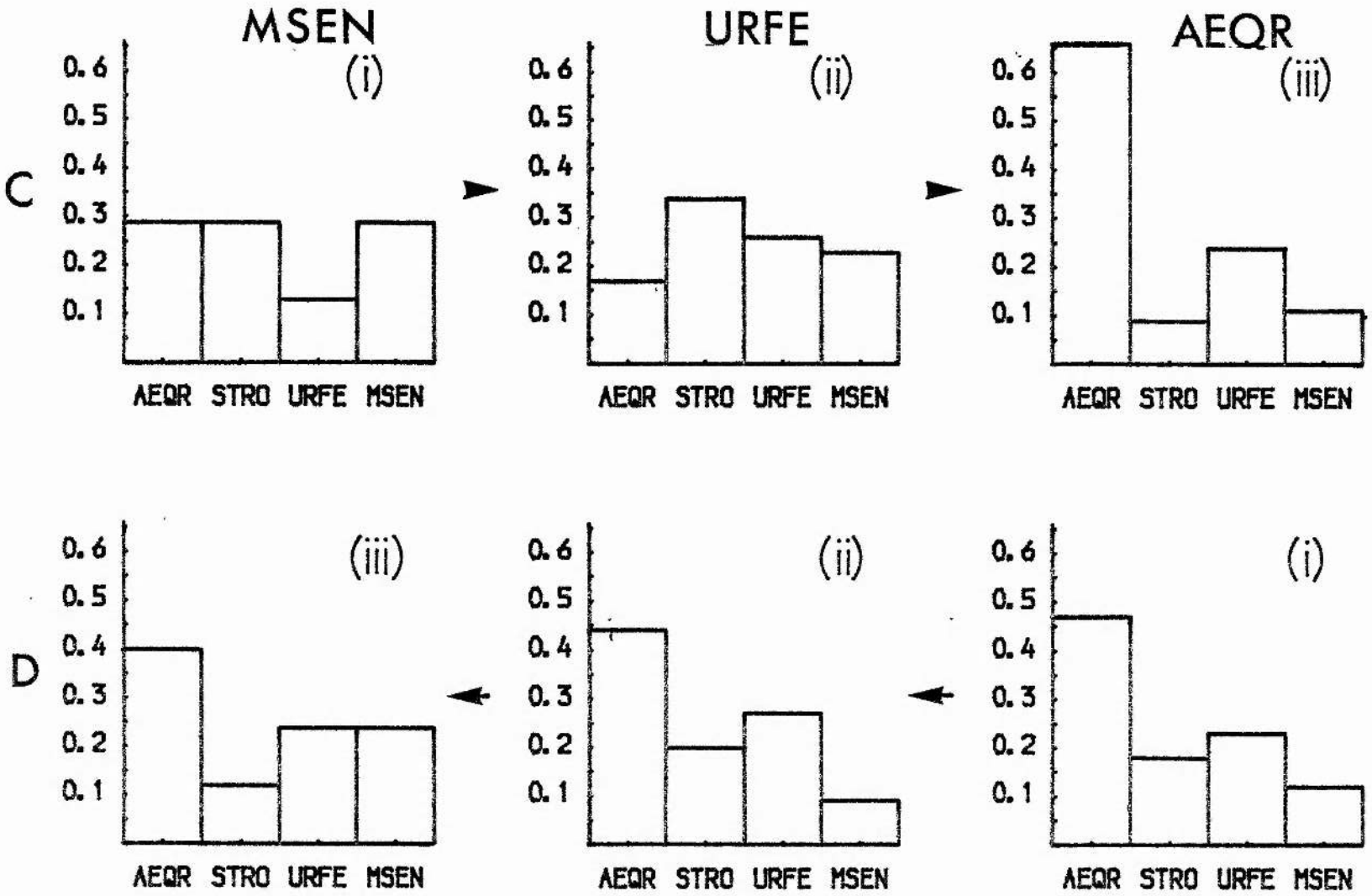

C
MSEN
(i)
URFE
(ii)
AEQR
(iii)
50
40
30
20
10
AEQR STRO URFE MSEN
D
(iii)
(ii)
(i)

Figure 11.

Histograms showing the proportion of trials in which each anemone species was selected under the three conditioning regimes of behavioural experiment 3(i).


C
MSEN
(i)
URFE
(ii)
AEQR
(iii)
0.6
0.5
0.4
0.3
0.2
0.1
AEQR STRO URFE MSEN
D
(iii)
(ii)
(i)

falls slightly from graphs (i) to (iii). Similarly, the pattern of response to the final diet remains low in graphs (i) and (ii) for both groups and rises after the final diet change. Over and above these features of the responses to individual anemone species, the molluscs appear to demonstrate an overall preference for A.equina; this was not indicated in previous experiments. Although the differences in response illustrated in Fig. 11 are in some instances small, the pattern of response is consistent. Greater replication would, undoubtedly, have resulted in better resolution in the data, but the emphasis in these latter experiments has been on the more preferred species. Despite these apparently small differences, the statistical analysis, based on the absolute data, shows clearly that a significant pattern of nudibranch responses obtains. Indeed it is this very consistency of response which has resulted in the acceptance of the statistical model M_2. It is reasonable, therefore, to consider these results as genuine support for the hypothesis concerning ingestive conditioning and past-history of diet as determinants of initial prey-selection response.

Behavioural Experiment 3(ii).

Tables 29 and 30 show the results of trials for groups E and F respectively. A total of 358 decisions were made out of a possible 450. The missing data are a result of the death of mollusc A4 during the course of the experiment and the failure of some nudibranchs to make a decision on some occasions.

Table 29. Results of trials undergone by Group E in Experiment 3(ii). (AEQR ► MSEN ► STRO).

		Conditioned to AEQR Mollusc						Conditioned to MSEN Mollusc						Conditioned to STRO Mollusc					
		A1	A2	A3	A4	A5	TOTAL	A1	A2	A3	A4	A5	TOTAL	A1	A2	A3	A4	A5	TOTAL
Chamber Entered	AEQR	7	4	4	1	2	18	7	4	4	7	3	25	10	3	5	1	4	23
	STRO	1	1	0	0	2	4	3	3	2	3	6	17	3	2	6	1	4	16
	URFE	2	1	2	1	6	12	0	2	2	0	1	5	1	0	1	0	3	5
	MSEN	0	1	2	0	1	4	1	2	3	1	1	8	1	6	2	0	2	11
	Blank	1	4	2	4	3	14	2	0	2	0	1	5	0	3	1	2	0	6
Trials when an anemone was chosen		10	7	8	2	11	38	11	11	11	11	11	55	15	11	14	2	13	55
Completed trials		11	11	10	6	14	52	13	11	13	11	12	60	15	14	15	4	13	61
Terminated trials		4	4	5	9	1	23	2	4	2	4	3	16	0	1	0	2+	2	5

2+ Denotes Mollusc died

Table 30. Results of trials undergone by Group F in Experiment 3(ii). (STRO ► MSEN ► AEQR).

		Conditioned to STRO Mollusc						Conditioned to MSEN Mollusc						Conditioned to AEQR Mollusc					
		B1	B2	B3	B4	B5	TOTAL	B1	B2	B3	B4	B5	TOTAL	B1	B2	B3	B4	B5	TOTAL
Chamber Entered	AEQR	4	1	4	4	6	19	4	3	1	1	3	12	3	5	3	9	3	23
	STRO	4	5	1	3	4	17	4	6	4	7	5	26	7	5	2	2	2	18
	URFE	2	1	3	0	0	6	1	0	4	0	2	7	1	1	0	0	1	3
	MSEN	3	0	0	0	1	4	4	2	3	3	1	13	4	2	1	2	2	11
	Blank	2	3	4	2	0	11	2	3	3	2	0	10	0	2	7	2	4	15
Trials when an anemone was chosen		13	7	8	7	11	46	13	11	12	11	11	58	15	13	6	13	8	55
Completed trials		15	10	12	9	11	57	15	14	15	13	11	68	15	15	13	15	12	60
Terminated trials		0	5	3	6	4	18	0	1	0	2	4	7	0	0	2	0	3	5

Table 31. Log max-likelihood values and likelihood ratio tests for Experiment 3(ii).

Model (M_x)	Log max-likelihood (L_x)	Likelihood Ratio $[2(L_{x+1} - L_x)]$
M_0	- 395.565	
		27.420 ***
M_1	- 391.899	
		88.694 N.S.
M_2	- 378.189	
M_3	- 333.842	

Conclusion: Model M_2 cannot be rejected.

The data have been analysed in an identical manner to behavioural experiment 3(i) and Table 31 shows the Log Max-Likelihood Ratios and the results of pairwise comparisons of more restricted models. These results are in agreement with behavioural experiment 3(i) and we thus conclude that conditioning diet and past history of diet, once again, influenced the pattern of response. Figs. 12 and 13, respectively, show the absolute and proportional selection frequency of anemone species under each of the three conditioning regimes. The patterns of response are similar to those for behavioural experiment 3(i) shown in Figs 10 and 11 although the responses to some anemone species are somewhat less consistent. Responses to S.troglodytes are notable in this respect; the proportional selection frequency unaccountably rises from E(i) to E(ii) (Fig. 13) following a change in conditioning diet from A.equina to M.senile. Furthermore, for group F (Fig. 13) selection of S.troglodytes increases (F(ii)) following a conditioning change from S.troglodytes to M.senile although it subsequently falls back on conditioning to A.equina (F(iii)).

Despite these inconsistencies, however, the broad patterns observed in behavioural experiment 3(i) (Figs. 10 and 11) can also be seen here. For example, following the responses to M.senile from F(i) to F(iii): the proportional selection frequency rises when the conditioning diet is changed from S.troglodytes to M.senile and then falls to an intermediate level in F(iii). Similarly, following responses to the initial

Figure 12.

Histograms showing the numbers of trials in which each anemone species was selected under the three conditioning regimes of behavioural experiment 3(ii).

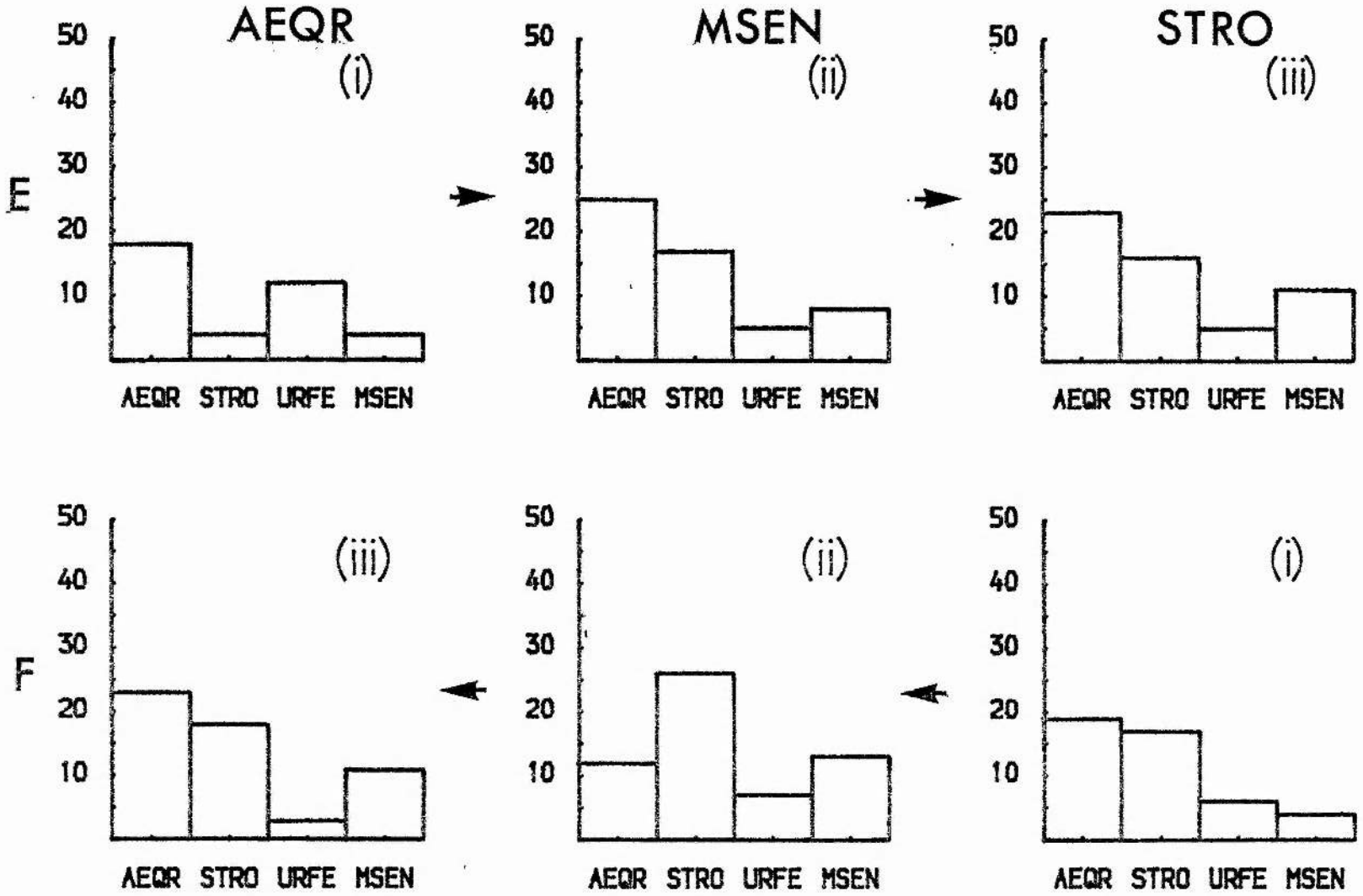
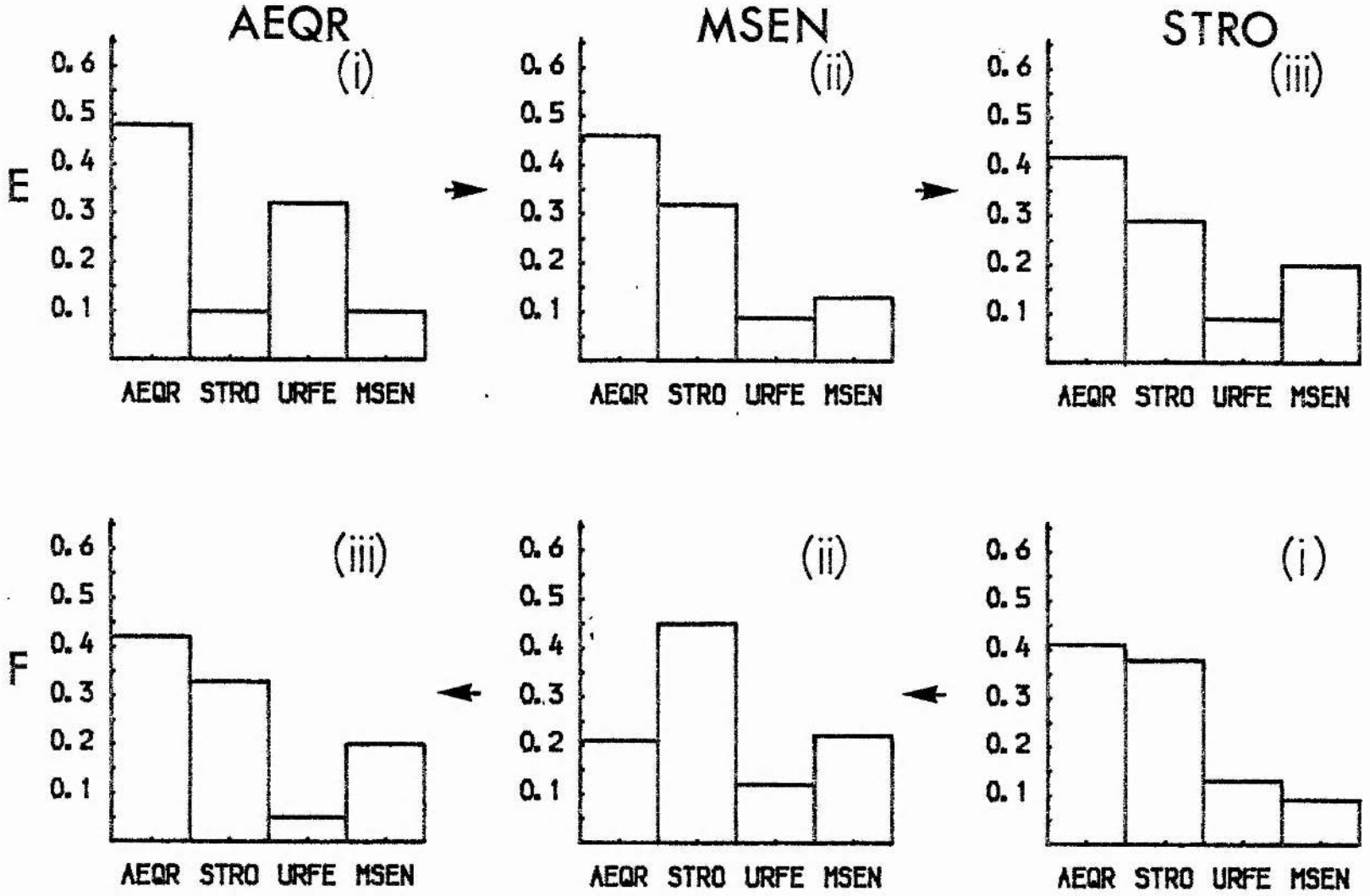

AEQR
(i)
MSEN
(ii)
STRO
(iii)
E
F
50
40
30
20
10
AEQR STRO URFE MSEN

Figure 13.

Histograms showing the proportion of trials in which each anemone species was selected under the three conditioning regimes of behavioural experiment 3(ii).


AEQR
(i)
MSEN
(ii)
STRO
(iii)
E
0.6
0.5
0.4
0.3
0.2
0.1
AEQR STRO URFE MSEN
(iii)
(ii)
(i)

conditioning diet (A.equina) in group E (Fig. 13): the proportional selection frequency is high in E(i), then falls slightly in E(ii) and yet further in E(iii). It should be noted, however, that this same pattern is not reflected by the absolute data (Fig. 12), whereby selection of A.equina rises in E(ii) and falls back slightly in E(iii). This is almost certainly due to the smaller number of completed trials (38 compared to 55) in E(i).

In summary, the results of experiments 3(i) and 3(ii) both support the conclusion that both present conditioning diet and past history of diet influence the prey selection responses of A.papillosa. Thus, nudibranchs feeding on the same diet may not select prey in similar proportions if their dietary histories differ. The marked differences in graphs (i) and (iii) between groups in each of the experiments (Figs. 11 and 13) clearly illustrate this point. By contrast in the only two cases in which the nudibranchs had experienced the same dietary history (i.e. C(iii) (Fig. 11) and E(i) (Fig. 13)) the responses are markedly similar with the exception of proportional responses to A.equina and U.felina.

GENERAL DISCUSSION OF THE THREE BEHAVIOURAL EXPERIMENTS

As previously outlined in the introduction to this chapter, a variety of experimental designs have been used to obtain data on prey-preferences in A.papillosa. A number of features of these studies detract somewhat from their utility. The most notable of these are:

(i) groups of nudibranchs were often used, thereby precluding the analysis of individual behavioural patterns.

(ii) in all cases, statistically adequate replication of choice experiments was somewhat lacking.

The present experimental design has necessitated considerable - but by no means prohibitive - replication, and has also permitted the monitoring of individual behaviour. Furthermore, there is no possibility of group-mediated modification of individual behaviour. Thus, the major sources of error, outlined above for previous investigations, have been largely circumvented in the present study. It is important to emphasise, however, that no reinforcement of choice, through feeding, could be permitted in these experiments and thus it is necessary to equate olfactory choice with subsequent feeding activity. Clearly, this may not in fact obtain although I am confident that, if permitted, subsequent prey contact would have been made.

The experiments have shown that ingestive conditioning plays an important part in determining the responses of A.papillosa when seeking prey items. Behavioural experiments 2 and 3 also demonstrate that past dietary history is an important determinant of individual nudibranch responses. These data indicate that ingestive conditioning results in a graded response to anemone species which is determined by the identity of recent dietary items and the order in which they were consumed. In view of this one might predict, on the basis of ingestive conditioning alone, a probabilistic model in which the most recently eaten prey item is most likely to be selected on the next occasion. Take an example in which a predator has a choice of three prey species (A, B and C). If the last eight meals were the sequence C,B,B,B,A,A,A,A the model would predict that species A is most likely to be selected on the next occasion. Species B is the second most probable choice and species C the least probable. This may be further complicated, however, because the quantities of each food species consumed are likely to have a bearing on the degree of conditioning. Furthermore, such predictions assume that in the absence of conditioning each anemone species is equally attractive to the predator. There is some indication that this may not be so: A.equina is often chosen by European A.papillosa and E.prolifera by its N.W. Pacific counterpart.

Clearly, the above predictions represent an oversimplification of the patterns of response that have been observed and to extend such 'predictions' to the field would be inappropriate. Despite this, however, the undoubted importance of ingestive conditioning to a given prey species, and its persistence over subsequent feeding encounters with other species, does suggest that a generalized pattern of responses is, perhaps, to be expected. Upon such patterns, a range of reactions to, as yet, unknown behavioural determinants must be superimposed before dietary choice can be completely specified.

Modification of a predator's behaviour as a result of ingestive conditioning, may be included within the general framework of training effects (e.g. Hughes,1979; McNair,1980,1981). Among invertebrates Landenberger (1968) for echinoderms and Murdoch (1969), Wood (1968), Pratt (1974), and Bayliss (1982) for gastropods, showed a training in preference after many prey encounters. By contrast, Meesters (1940) showed training in fish after only one encounter. The experiments presented here give no clear information on the number of prey encounters required to modify predatory behaviour. Prey preferences can only be determined through replicated trials over an extended period, during which time it is necessary to feed the nudibranchs. There is, however, no indication in any of the experiments that the pattern of response changes during the trials periods, and thus, it seems likely that a change in maintenance diet for five days is sufficient to elicit an

effect.

The selective advantage of ingestive conditioning, as demonstrated for A.papillosa is, as yet, unknown although a number of possible explanations may be suggested. Considerations arising from optimal foraging theory provide a valuable framework within which to discuss hypotheses regarding the ingestive conditioning phenomenon.

Standard optimal foraging theory predicts that in an optimal diet, i.e. one that maximises net energy intake per unit of foraging time (conventionally, E/h), an increase in total prey abundance cannot broaden the optimal diet to include less profitable species or individuals. Thus, increased prey abundance either narrows the diet or has no effect. Fundamental optimal diet models make no provision for the effects of experience on the foraging behaviour of the predator. Searching methods and the efficiency of prey location, evaluation, and handling time, are all assumed to be constant, as is the assimilation efficiency for a given diet. If, as a result of training effects, the predator is able to improve its ability to locate, handle or assimilate a given diet, then E/h or "profitability" of that diet would increase.

Recently, McNair (1980,1981) has considered the implications of training effects (particularly 'search image' formation) on the behaviour of model predators. He has indicated that the inclusion of low profitability prey in an optimal diet may be

permissible if training occurs. In a similar vein, Hughes (1979) incorporated training into an optimal diet model by allowing handling time to be a function of the encounter rate with a particular prey.

Ingestive conditioning functions as a mechanism whereby foraging effort is concentrated upon the few prey species most recently eaten. It is, perhaps, reasonable to suppose that such concentration of effort may facilitate the 'training' alluded to above, and that, as a result of such training, E/h values for the species concerned may be elevated. Thus, ingestive conditioning may facilitate an improvement in overall foraging efficiency by concentrating effort on a limited range of prey species.

A number of alternatives exist regarding the mechanism by which E/h values may be improved. With particular reference to the present study three major possibilities exist:

i) The formation of an olfactory search image may contribute to an elevation in foraging efficiency by screening out 'noise' in a complex environment.

ii) By learning to cope more effectively, by experience, with a given anemones defensive mechanisms, handling efficiencies may be improved.

iii) Quantitative and/or qualitative changes in digestive enzyme production, in response to a given diet, may render it

advantageous to remain on a species to which the digestive physiology is already adapted.

From the results shown for behavioural experiment 1 (Table 22), and from further observations from experiments 2 and 3, no evidence can be provided to substantiate hypothesis (i) above. There is, as yet, no direct evidence for the formation of olfactory search images although Fauchald & Jumars (1979) do suggest that this may occur in phyllodocid polychaetes.

Hypotheses (ii) and (iii) remain untested for A.papillosa and any comments on their respective merits, as explanations of the observed phenomenon, would be purely speculative. A number of studies have shown, however, that such mechanisms operate for other species. For example, Morgan (1972) showed that handling times for predation on the mussel Mytilus edulis (L.) by the dog-whelk Nucella lapillus (L.), were reduced when the predator had experience of the prey. Similarly, Edwards (1975) showed that the naticid snail Polinices duplicatus (Say) feeds at a faster rate on the clam Mya arenaria (L.) after experience of that prey species. Increased handling efficiencies, by more experienced predators, has also been demonstrated by Lawton et al. (1974) for the insect Notonecta glauca (L.).

A number of studies have also shown adaptations of digestive physiology in response to diet changes. The studies of Prosser et al. (1958) and van Weel (1959) for the same terrestrial gastropod are, perhaps, of most relevance in the present case.

These two studies demonstrated qualitative and quantitative differences in digestive enzymes when molluscs were fed on either protein- or carbohydrate-rich diets. Another possible advantage of ingestive conditioning has been proposed by Edmunds(1983) who suggests that ingestive conditioning results in a more effective camouflage for A.papillosa (as a result of pigment changes) and hence reduces the possibility of its being attacked by predatory fish. This is based on the observation that (juvenile) A.papillosa tend to assume the colour of their anemone prey. My own observations indicate that while this is certainly true in some instances (especially for red A.equina), prey-derived colouration becomes subordinate to the molluscs' melanism as the nudibranch grows. The result is that adults are of a comparatively uniform grey-brown colour. Furthermore, any such differences in colour for nudibranchs feeding on most of the species in this study are unlikely to provide markedly different degrees of protection from attack. Despite these reservations, nudibranchs feeding on M.senile are somewhat paler than their counterparts on other diets and this colouration may provide some protection for individuals feeding in clones of this anemone.

Thus far, discussion of the adaptive significance of ingestive conditioning has taken no account of the possible effects of variations in the abundance of prey species in the field. It is reasonable to suppose that such variations in abundance will play a major role in determining the behavioural patterns of the predator.

Settlement by nudibranch veliger larvae is the first point at which the differential abundance of prey might have an effect. For the large proportion of nudibranch species investigated the definitive stimulus for the competent veliger to settle and metamorphose is the presence of the live adult diet (see Todd,1981 for review). The same probably applies to A.papillosa and since the animal is capable of feeding on a wide variety of anemone species it is likely that all species investigated here are capable of inducing metamorphosis. If this is so - and in the absence of any marked specificity - then in a given locality, by probability, the majority of larvae will settle on the most abundant acceptable anemone species. If, through training, the E/h value for the most abundant anemone is thereby elevated to become the most valuable of all the species available, the overall foraging efficiency would be improved by remaining on that prey species. This could only occur if the magnitude of change in E/h, through training, is greater than the absolute difference in E/h between prey species, if training did not occur. This assumption is, perhaps, most likely to hold if the overall differences in prey-values, without training, are small. Should training effects obtain it may be advantageous for A.papillosa to preferentially seek out the most abundant prey species. Ingestive conditioning would result in an increased probability that the species taken in any given meal will be the same as that taken in the previous meal; thus, it may provide a mechanism for such behaviour. This has been formally demonstrated by Murdoch (1969) who shows that frequency-dependent

prey selection can occur when training results in an increased preference for a species.

Although prey selection by A.papillosa can be largely accounted for in terms of dietary history, it is clear that conditioning effects are by no means exclusive; species that, as far as I am aware, had never been eaten by the nudibranchs were still frequently selected (e.g. in behavioural experiment 1, another species was selected on 85 occasions compared to 68 for S.troglodytes-conditioned animals). This plasticity of prey response may permit a continual monitoring of the predator's environment in order to accomodate fluctuations in prey species abundance. This, in itself, would result in attack on respective prey species as they become sequentially abundant, but it should also be noted that similar fluctuations in prey relative abundances may also arise from biotic and abiotic factors other than A.papillosa predation.

Few published accounts consider the problem of how a predator 'samples' its environment, despite this being an implicit prerequisite of most foraging models. One notable exception, which relates to the situation for A.papillosa, is provided by Krebs et al. (1978) who considered the problem of choosing the optimal balance between exploration and exploitation of two different feeding patches by the bird Parus major (L.).

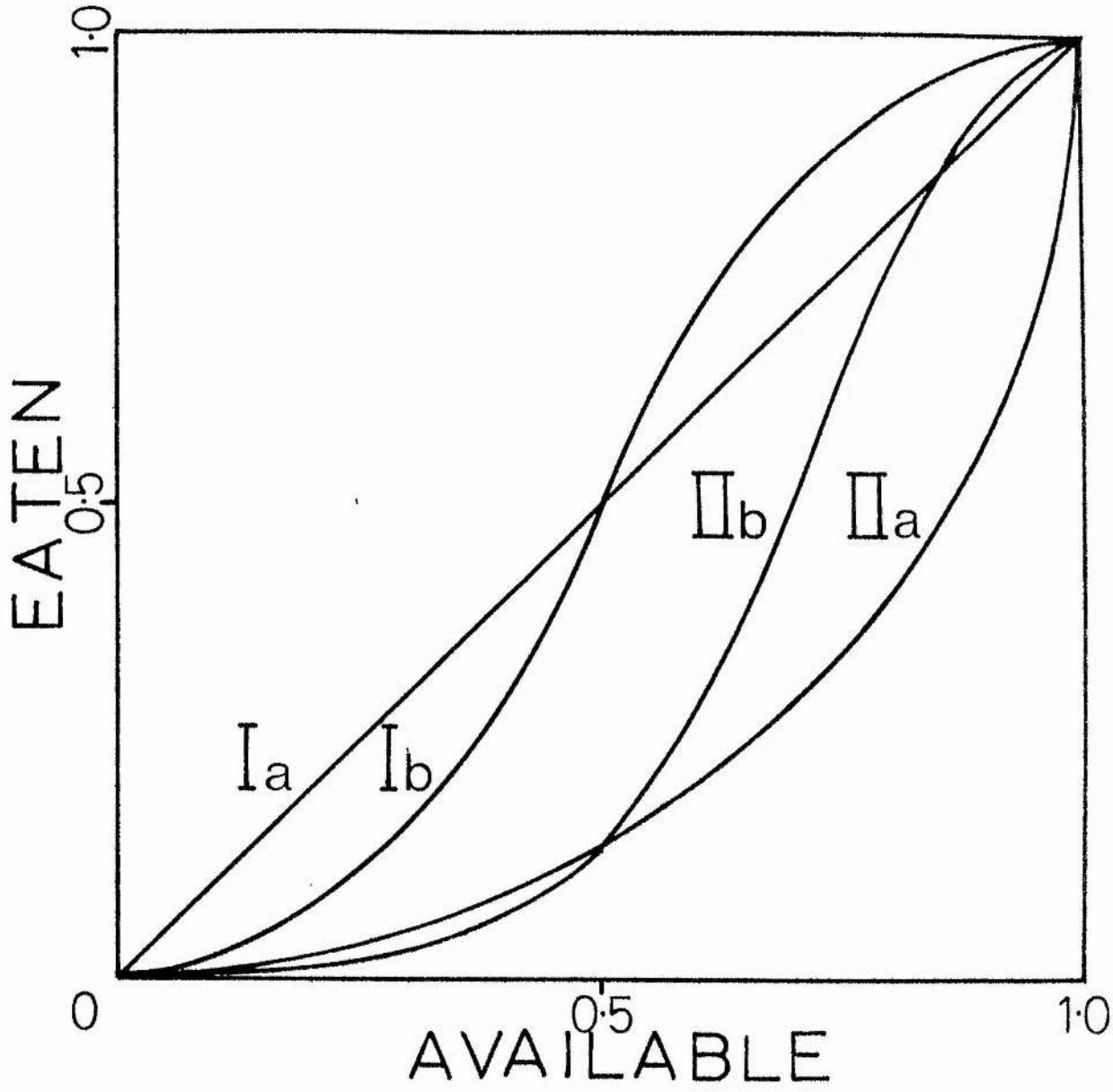
It has been stated above that ingestive conditioning may represent a mechanism whereby the most abundant species in the predator's habitat is concentrated upon, while the rare are ignored. Such frequency-dependent prey selection is termed "switching" (Elton,1927; Murdoch,1969). Formally defined, switching implies that as the availability of prey i increases (or decreases) its fractional representation in the total diet increases (or decreases) faster than the fraction it constitutes of the total prey availability (Murdoch,1969; Murdoch et al.,1975). Fig. 14 illustrates this behaviour graphically and shows that switching is not restricted to situations where there is equal preference for the two prey species when they are equally abundant. Switching behaviour, or apostatic selection (which refers to such responses to different morphs of the same prey species), has been demonstrated experimentally for a wide range of organisms. These include birds (Allen,1972,1974), fish (Murdoch et al.,1975), parasitoids (Cornell & Pimental,1978), and gastropods (Murdoch,1969). This behaviour has also been extensively considered in theoretical contexts (e.g. Elton & Greenwood,1970; Fullick & Greenwood,1979; Lawton et al.,1974; Murdoch & Oaten,1975; Commins et al.,1976). Experimental evidence for the adaptive significance of switching is, however, noticeably lacking. Hubbard et al. (1982), have suggested that there is a clear empirical and biological link between apostatic prey-selection (the theory applies equally to switching) and optimal foraging theory, in which the predator is assumed to maximise its inclusive fitness. In its most general form this

Figure 14.

Switching behaviour in a system with two prey-species. The ordinate represents the proportional availability of one of the prey species while the abscissa shows the proportion of the same species in the predator's diet.

Ia and IIa show the patterns of predation in the absence of switching, for a predator which shows no preference at equality (Ia), or a five-fold preference at equality for one of the species (IIa).

Ib and IIb show switching behaviour for a predator which displays either no preference at equality (Ib) or a five-fold preference at equality for one species (IIb).


1·0
0·5
EATEN
0
0·5
1·0
AVAILABLE
Ia
Ib
IIb
IIa

link is dependent on frequency-dependent changes in prey-values. In the case of A.papillosa, this may be effected by any or all of the three hypotheses outlined earlier in this discussion; notably, olfactory search image formation, improved prey handling times with experience, or adaptation of digestive physiology.

Clearly, the experiments in this chapter do not demonstrate switching behaviour in A.papillosa. It is reasonable to suppose, however, that ingestive conditioning may represent a mechanism by which switching behaviour is effected and such may extend to the present predator-prey associations. The experiments described in the subsequent chapter test for switching in A.papillosa with two anemone species.

Chapter 6.

AN INVESTIGATION OF SWITCHING BEHAVIOUR IN A.PAPILLOSA.

INTRODUCTION.

The objective of the experiments described in this chapter was to test for "switching" behaviour (sensu Murdoch,1969) in A.papillosa at prey densities and relative prey abundances that the nudibranch might reasonably be expected to encounter in the field. As discussed in the previous chapter, switching implies that as the availability of prey i increases (or decreases) its fractional representation in the total diet increases (or decreases) faster than the fraction it constitutes of the total prey availability (Murdoch,1969; Murdoch et al.,1975).

MATERIALS AND METHODS.

In all switching experiments only two prey species were on offer, notably the red morph of A.equina, and S.troglodytes. These species were chosen for two reasons: first, they have both been shown to exert a strong ingestive conditioning effect (behavioural experiment 1), and secondly, small, comparably sized individuals of both species may be locally obtained relatively easily. Before the experiment commenced anemones were placed in cleaned mussel shells and allowed to attach. This was to prevent nudibranchs from attacking detached anemones through the pedal

disc and also to facilitate operator handling.

The experiments were conducted with six nudibranchs which were maintained throughout in individual 2.5l plastic containers in a seawater cascade. Before the experiment started the nudibranchs were fed on U.felina to ensure that none of the animals were conditioned to either of the prey species on offer. The six animals were divided into two replicate groups of three and every nudibranch in each group was presented with a different ratio (comprising ten individuals) of the two prey species. The ratios of S.troglodytes:A.equina presented were 7:3, 5:5, and 3:7. In order to maintain the correct ratio of anemone species for each nudibranch, and to record the patterns of consumption, all nudibranchs were inspected daily. Consumed or partially eaten anemones were replaced with another individual of the same species. Seawater temperature was also recorded daily. Any anemones which had detached from their mussel shells, and had not re-attached to the container, were replaced.

A series of three experiments was conducted within this basic methodology, the latter two experiments being initiated in the light of the initial results. The three experiments are summarised in the following schedule:

Experiment 1. (22.11.82 to 22.02.83 inclusive)

Individuals in both triplicate groups were maintained on their respective prey ratios for 47d (22.11.82 to 06.01.83) (experiment 1(1)) and then changed around so that each nudibranch experienced a new prey ratio (experiment 1(2)).
The ratios were changed on the following manner:

Nudibranchs previously feeding on 7:3 (Stro:Aeqr) changed to 5:5
Nudibranchs previously feeding on 5:5 (Stro:Aeqr) changed to 3:7
Nudibranchs previously feeding on 3:7 (Stro:Aeqr) changed to 7:3

The experiment was run for a further 47d (06.01.83 to 22.02.83) offering the second ratio. The net effect of the experimental design was that each individual was offered two different ratios and thus, within each duplicate group, there are two replicates for each prey ratio.

Experiment 2.

All nudibranchs were maintained on the ratio they experienced in experiment 1(2) except that anemones were not replaced as they were eaten. When a nudibranch had eaten all ten anemones the ratio was restored and feeding with replacement was recorded for a further 47d.

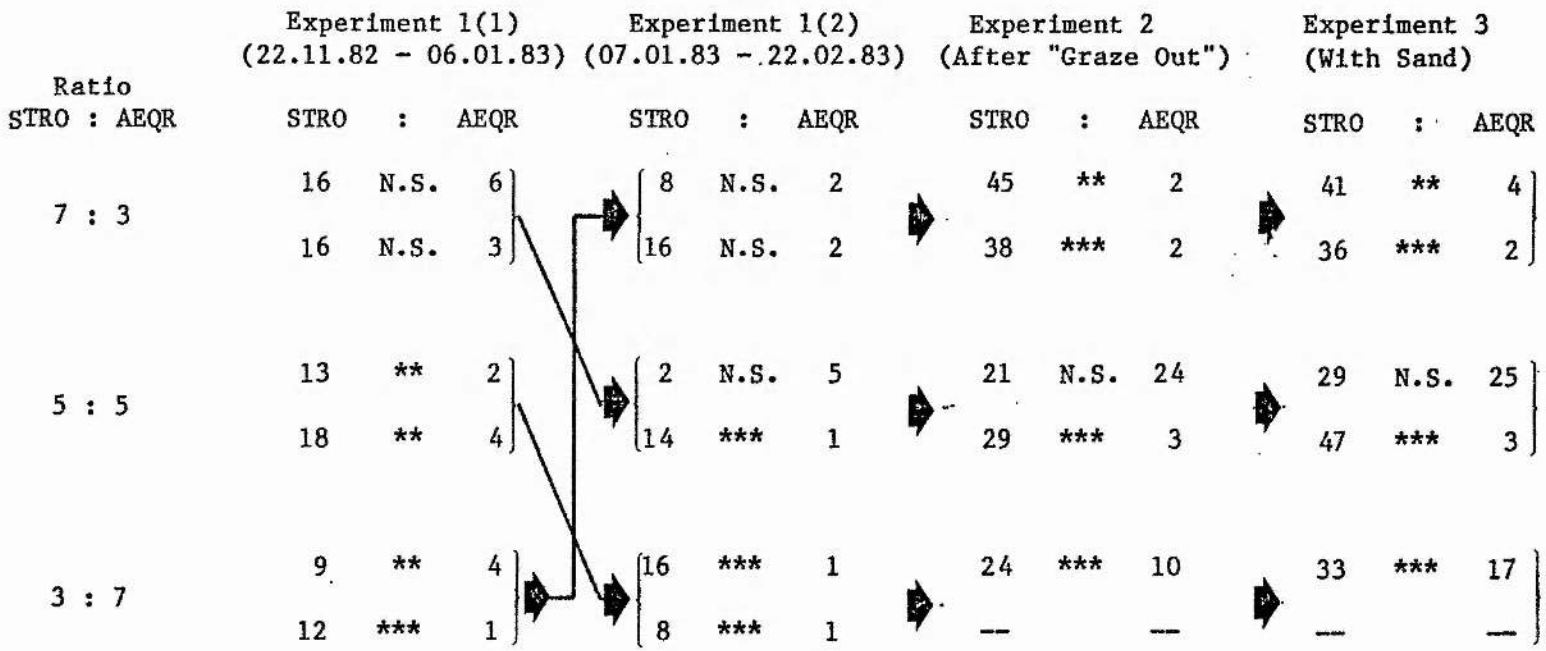
Experiment 3.

The ratios offered to the nudibranchs in experiment 2 were maintained but sand was added to the bottom of each container. S.troglodytes attached to mussel shells were then buried in the sand with only the tentacle crown protruding; A.equina were left on the surface. This experiment attempts to mimic the burial of S.troglodytes observed in the field and investigates its effect on predation responses. The experiment was once again run for 47d.

RESULTS.

Table 32 shows the numbers of each species eaten in each of the experiments. The replicates in which the numbers eaten depart significantly from those that would be expected if anemones were eaten ad hoc are also shown in this table. Significant differences were tested for using chi-squared tests. One notable feature of these data as a whole is the general increase in the numbers eaten during later experiments. This is almost certainly due to the warmer seawater temperatures following the cold winter. One individual died before experiment 2 was initiated: this individual could not be replaced.

Table 32. The numbers of anemones eaten in each of the switching experiments. The arrows follow the treatment of individual nudibranchs



Asterisks denote conventional significance levels.

Tables 33 to 35 show the sequence of anemone selections by the nudibranch in each of the switching experiments: the increased feeding rates with temperature alluded to above are also shown. It can be seen from the sequence of selections shown in the tables that, when the less preferred species (usually A.equina) was selected it was not usually eaten in consecutive meals. The conditioning effect from consuming a single anemone does not, therefore, appear to be sufficient to induce further selections of that species.

Fig. 15 presents the complete data set graphically - the numbers of each species eaten for each histogram being expressed as a proportion of the total eaten in that replicate. The arrows both in Table 32 and Fig. 15 indicate the treatments experienced by individual nudibranchs.

The results of the chi-squared tests show that, for experiment 1, three of the four nudibranchs presented with the two species in equal proportions showed a significant preference for S.troglodytes. The remaining nudibranch showed no statistically significant preference for either species; although A.equina was selected more often, the total number of anemones eaten during this period, however, was only seven compared with 15 for the other mollusc at this ratio. With so few anemones taken even an outcome of six A.equina to one S.troglodytes would not be statistically significant.

Table 33. A summary table showing the order in which anemones were selected in switching experiments 1(1) and 1(2).

Experiment 1(1)

Ratio (STRO:AEQR)	7:3	7:3	5:5	5:5	3:7	3:7
	5 STRO	7 STRO	2 STRO	1 AEQR	1 AEQR	3 STRO
	1 AEQR	1 AEQR	1 AEQR	1 STRO	1 STRO	1 AEQR
	3 STRO	1 STRO	8 STRO	1 AEQR	3 STRO	9 STRO
	3 AEQR	1 AEQR	1 AEQR	1 STRO	1 AEQR	
	7 STRO	8 STRO	2 STRO	1 AEQR	1 STRO	
	1 AEQR	1 AEQR	1 STRO	9 STRO	1 AEQR	
	1 STRO			1 AEQR	3 STRO	
	1 AEQR			7 STRO	1 AEQR	
					1 STRO	
Anemones eaten d^{-1}	0.39	0.41	0.33	0.48	0.28	0.28
Mean temperature (^{o}C)	6.2					
Max temperature (^{o}C) (with date)	8.0 (01.12.82)					
Min temperature (^{o}C) (with date)	4.0 (18.12.83)					

Experiment 1(2)

Ratio (STRO:AEQR)	7:3	7:3	5:5	5:5	3:7	3:7
	1 STRO	1 STRO	2 AEQR	5 STRO	1 STRO	2 STRO
	1 AEQR	1 AEQR	1 STRO	1 AEQR	1 AEQR	1 AEQR
	7 STRO	10 STRO	1 AEQR	9 STRO	15 STRO	6 STRO
	1 AEQR	1 AEQR	1 STRO			
		5 STRO	2 AEQR			
Anemones eaten d^{-1}	0.22	0.39	0.15	0.33	0.37	0.19
Mean temperature (^{o}C)	5.1					
Max temperature (^{o}C) (with date)	7.1 (17.01.83)					
Min temperature (^{o}C) (with date)	3.0 (12.02.83)					

Table 34. A summary table showing the order in which anemones were selected in switching experiment 2 (after "Graze Out").

Ratio (STRO:AEQR)	7:3	7:3	5:5	5:5	3:7	3:7
	7 STRO	9 STRO	1 STRO	11 STRO	1 STRO	
	1 AEQR	1 AEQR	1 AEQR	1 AEQR	2 AEQR	
	26 STRO	20 STRO	2 STRO	14 STRO	1 STRO	
	1 AEQR	1 AEQR	1 AEQR	1 AEQR	1 AEQR	
	12 STRO	9 STRO	2 STRO	2 STRO	1 STRO	
			5 AEQR	1 AEQR	3 AEQR	
			1 STRO	2 STRO	12 STRO	
			1 AEQR		2 AEQR	
			2 STRO		7 STRO	
			1 AEQR		1 AEQR	
			1 STRO		2 STRO	
			1 AEQR		1 AEQR	
			1 STRO			
			2 AEQR			
			1 STRO			
			3 AEQR			
			2 STRO			
			2 AEQR			
			1 STRO			
			2 AEQR			
			1 STRO			
			2 AEQR			
			3 STRO			
			1 AEQR			
			2 STRO			
			2 AEQR			
			1 STRO			
Anemones eaten d^{-1}	1.02	0.87	0.98	0.69	0.74	
Mean temperature (^{o}C)	8.5	7.4	6.4	6.4	8.5	
Max temperature (^{o}C)	10.9	9.5	8.0	8.0	10.9	
Date (1983)	(24.05)	(20.05)	(30.04)	(30.04)	(24.05)	
Min temperature (^{o}C)	6.3	5.4	4.8	4.8	6.3	
Date (1983)	(18.04)	(04.04)	(03.04)	(03.04)	(18.04)	

Table 35. A summary table showing the order in which anemones were selected in switching experiment 3 (with sand).

Ratio (STRO:AEQR)	7:3	7:3	5:5	5:5	3:7	3:7
	13 STRO	29 STRO	10 STRO	15 STRO	1 STRO	
	1 AEQR	2 AEQR	1 AEQR	1 AEQR	1 AEQR	
	3 STRO	7 STRO	4 STRO	5 STRO	3 STRO	
	1 AEQR		1 AEQR	1 AEQR	1 AEQR	
	4 STRO		2 STRO	12 STRO	1 STRO	
	1 AEQR		2 AEQR	1 AEQR	1 AEQR	
	9 STRO		1 STRO	15 STRO	1 STRO	
	1 AEQR		1 AEQR		1 AEQR	
	11 STRO		4 STRO		1 STRO	
			1 AEQR		1 AEQR	
			1 STRO		1 STRO	
			4 AEQR		4 AEQR	
			2 STRO		3 STRO	
			2 AEQR		1 AEQR	
			1 STRO		2 STRO	
			7 AEQR		1 AEQR	
			1 STRO		2 STRO	
			4 AEQR		1 AEQR	
			2 STRO		3 STRO	
			2 AEQR		1 AEQR	
			1 STRO		6 STRO	
					1 AEQR	
					3 STRO	
					1 AEQR	
					4 STRO	
					2 AEQR	
					2 STRO	
Anemones eaten d^{-1}	0.98	0.83	1.17	1.09	1.09	
Mean temperature (^{o}C)	12.39	11.5	11.4	11.4	12.2	
Max temperature (^{o}C)	16.0	14.5	13.8	13.8	16.0	
Date (1983)	(15.07)	(06.07)	(22.05)	(22.05)	(15.07)	
Min temperature (^{o}C)	9.0	9.2	9.0	9.0	9.0	
Date (1983)	(03.06)	(29.05)	(19.05)	(19.05)	(03.06)	

Figure 15.

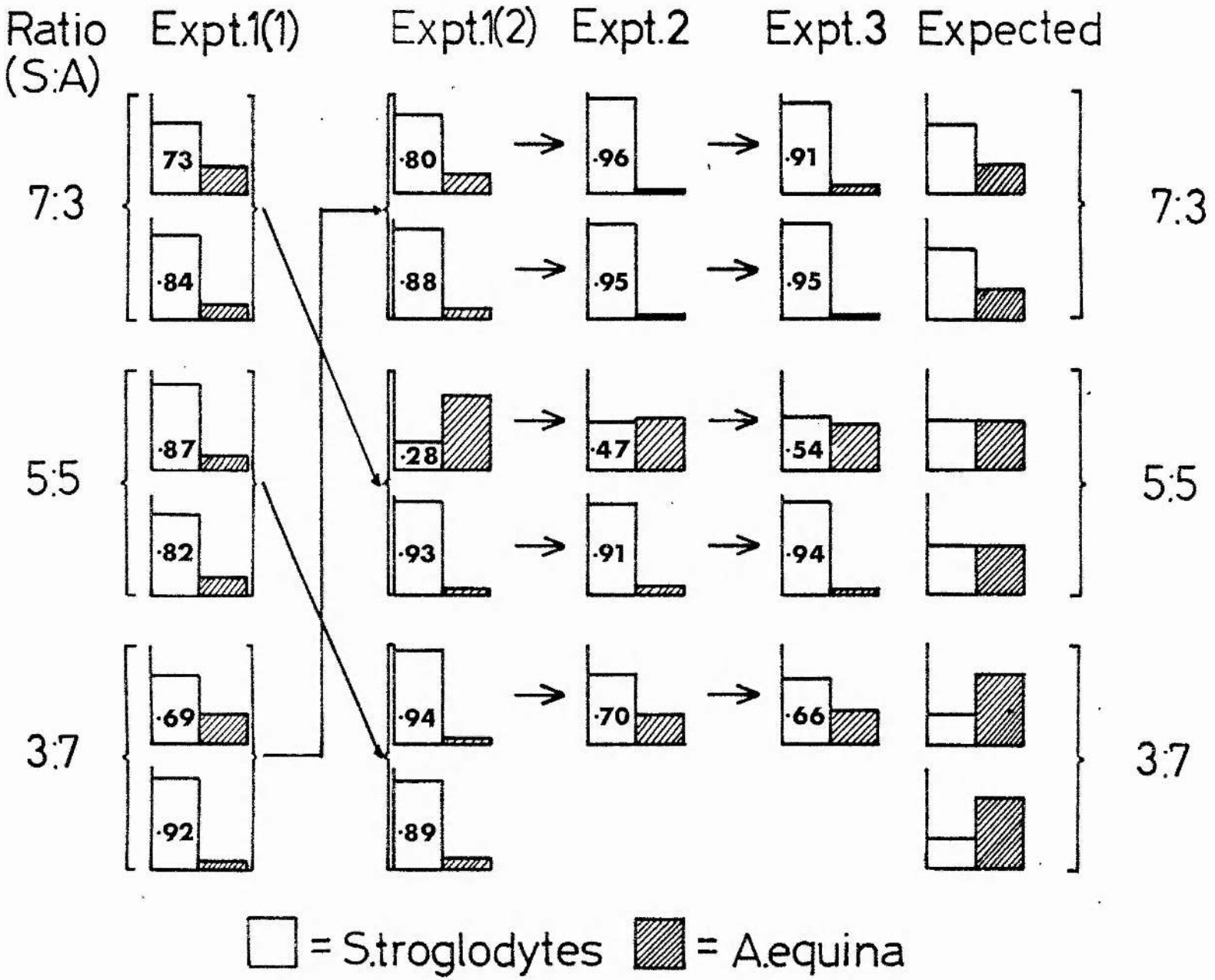
The proportion of A.equina and S.troglodytes selected by each nudibranch encountering the three prey ratios in each of the switching experiments. The corresponding numerical data are presented in Table 32.

Experiment 1(1). Initial prey ratio.

Experiment 1(2). Subsequent prey ratio.

Experiment 2. Restored ratio as in experiment 1(2) following graze-out.

Experiment 3. Restored ratio in experiment 2 with S.troglodytes buried.


Ratio (S:A)
Expt.1(1)
Expt.1(2)
Expt.2
Expt.3
Expected
7:3
5:5
3:7
.73
.84
.80
.88
.96
.95
.91
.95
7:3
.87
.82
.28
.93
.47
.91
.54
.94
5:5
.69
.92
.94
.89
.70
.66
3:7
□ = S.troglodytes
▨ = A.equina

For nudibranchs presented with a 7:3 ratio (S.troglodytes:A.equina) in experiment 1 the species were taken in proportion to their relative abundances. This pattern does not, however, hold for nudibranchs presented with a ratio of 3:7 (Stro:Aeqr), where a strong preference for S.troglodytes was shown.

The pattern which emerges from these results is one of an overall preference S.troglodytes, with little detectable change in behaviour that could be attributable to prey relative abundance. The absence of a preference for S.troglodytes in the 7:3 (Stro:Aeqr) ratio is difficult to account for in view of the strong preference demonstrated when the ratio was reversed to 3:7. This may be attributable to the relatively low numbers of anemones eaten in relation to the numbers of the most abundant anemone that would be required to be eaten before a significant preference could be detected.

The results of this experiment clearly show that switching behaviour did not occur. This may have been due to the experimental system not resulting in the nudibranchs becoming conditioned to the most abundant prey-species at inequality. Such a situation may simply be a consequence of the physical environment; the predator would presumably have encountered both prey species frequently during the experiment because the rarer of the two species was still quite common in absolute terms. Thus, the small homogeneous environment may not have produced the

gradual increase in the probability of attack upon the common species. Such an explanation for the absence of switching in an experimental system such as this was first advanced by Murdoch (1969) in studies on the predatory gastropod Acanthina spirata (Blainville).

In order to test this possibility the nudibranchs were required to eat all ten anemones in their respective treatments at the end of experiment 1(2) without replacement, thereby simulating the total consumption of the prey items from a localized 'patch' of the predator's habitat. It was predicted that such a 'graze-out' should result in the conditioning of the nudibranchs to the most abundant species and thus, switching behaviour on encounter with a new 'patch' in which the same prey ratios were restored.

The enforced conditioning (in experiment 2), through eating all the anemones in the 'patch' without replacement, did effect some changes, compared to experiment 1, in the pattern of consumption. The two nudibranchs presented with 7:3 (Stro:Aeqr) showed a change from no preference in experiment 1 to a statistically significant preference for S.troglodytes following the 'grazing-out' period. This is presumably a result of the conditioning of the nudibranchs to the most abundant species in the 'patch'. A similar, but non-significant, effect can be seen in the case of the single nudibranch feeding on the ratio 3:7 (Stro:Aeqr); this showed a marked increase in the relative numbers of A.equina selected although S.troglodytes was still

preferred (see Fig. 15). However, where prey were equally abundant the two nudibranchs showed similar patterns to those observed in experiment 1 (Fig. 15). This is, perhaps, unsurprising since equal numbers of both species were present at the start of 'grazing out' period and thus, no strong conditioning to either species would occur.

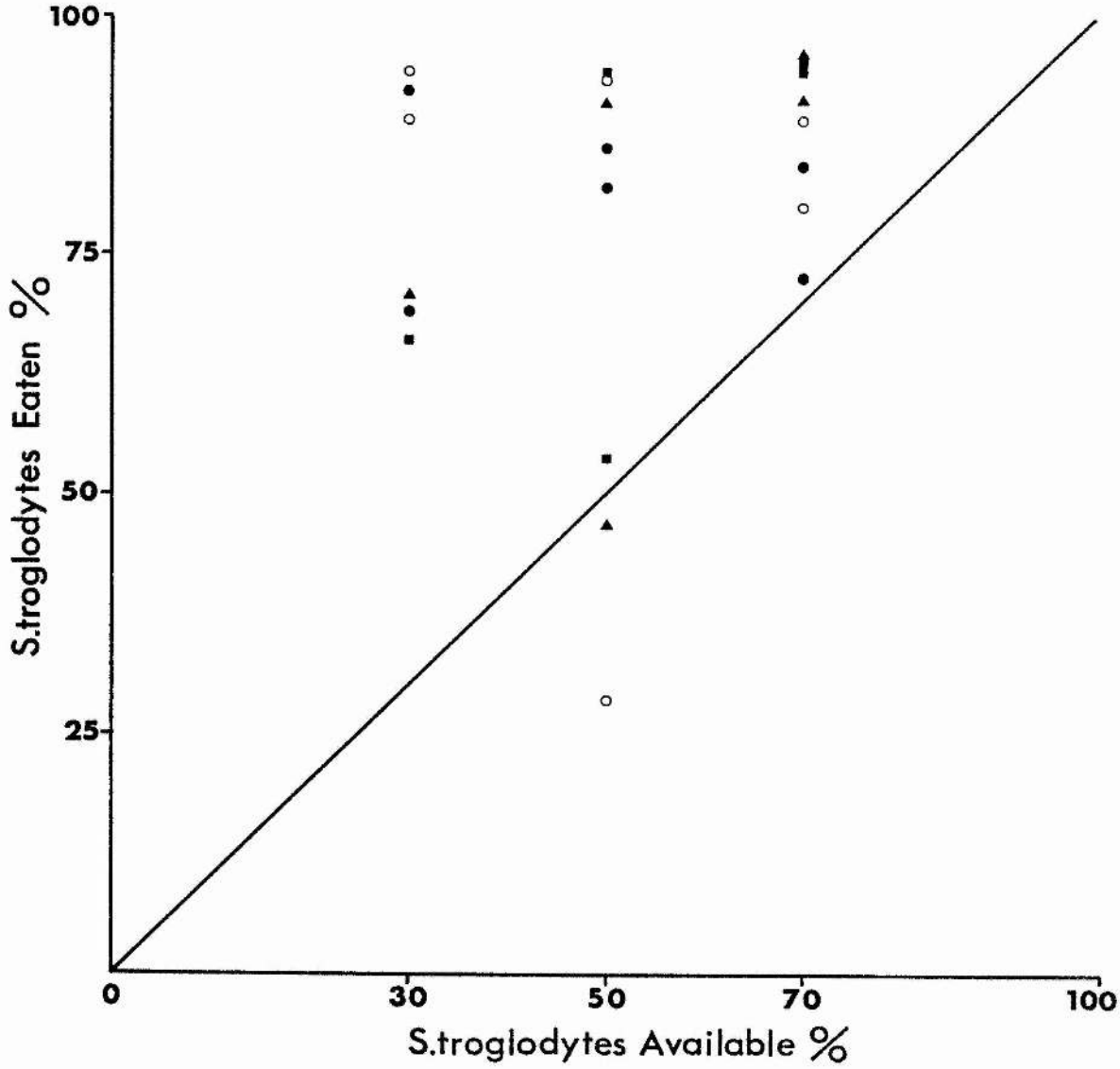
It is clear from the results of experiment 2 that, despite the enforced conditioning effect, the nudibranchs did not demonstrate switching.

The results for experiment 3 (in which S.troglodytes were buried) differ only slightly from those in experiment 2 in that there is still a marked preference for S.troglodytes. It thus appears that, for S.troglodytes, burial in sand to a depth of 1-1.5cm has little or no effect on the detection and predation patterns demonstrated by the nudibranchs. Thus the efficacy of burial by S.troglodytes as a deterent to predation is still open to question.

The general absence of any switching behaviour in A.papillosa is illustrated in Figure 16 which shows the percentage of S.troglodytes, eaten by all nudibranchs in each experiment, in relation to the percentage of S.troglodytes available. The line with a slope of 1.0 that is drawn through the graph indicates the consumption level that is proportional to prey relative abundance. Points falling above the line indicate a preference for S.troglodytes, while points below the line

Figure 16.

The percentage of S.troglodytes in the diet in relation to the percentage of S.troglodytes available to the nudibranchs. For explanation see text.



• Expt. 1(1); ○ Expt. 1(2); ▲ Expt. 2.; ■ Expt. 3.

indicate a preference for A.equina. If switching behaviour had occurred, points would fall below the line (i.e. preference for A.equina) when only 30% of the available prey were S.troglodytes. This analysis is complicated somewhat, however, when a preference for one or other species is shown at equality. In such a case switching can still occur but the line representing the no-switching condition is curved (see Fig. 14). Murdoch (1969) used a different graphical representation in which the proportions of available and eaten prey were calculated. This method has the advantage that the no-switching condition is always a straight line passing through the preference at equality. In the present case, however, the preference at equality is very variable and the more easily understood percentage axes have been chosen to illustrate the absence of switching more clearly.

Thus far it has been assumed that in the experimental system individuals of both prey-species are attacked and consumed with equal ease. There is some evidence to suggest, however, that this may not be the case. Observations from experiment 1 indicated that A.equina detached from the mussel shells much more often than S.troglodytes, and that they failed to re-attach: furthermore, many of these detached individuals were also slightly inflated indicating that they had, indeed, been attacked by A.papillosa. To investigate this further the number of anemones found detached was recorded during the course of experiment 2.

A total of 19 A.equina were found detached in experiment 2. The distribution of these detachments between the prey ratios is shown below:

Ratio (Stro:Aeqr)	7:3	5:5	3:7
Replicate 1	2	8	1
Replicate 2	3	5	

No detachment by S.troglodytes was observed.

Detachment and column inflation are well documented escape responses for A.equina (Edmunds et al.,1974), and thus, the detachments observed in this experiment may represent succesful escape behaviour in response to attack by A.papillosa. If this is the case, then the two prey-species are not equally available, and the patterns observed may result from preferences determined by a greater probability of a succesful attack on S.troglodytes.

DISCUSSION.

The objective of this series of experiments was to test the hypothesis that, under laboratory conditions, A.papillosa would demonstrate switching behaviour. None of the experimental results support this hypothesis. A strong overall preference for S.troglodytes was demonstrated by all but one nudibranch for most of the experiments and this preference was apparently unaffected

by the relative abundances of the prey species presented. These results are consistent with those of Murdoch (1969) who reported that predators with strong preferences did not show switching.

It is possible that under different experimental circumstances (e.g. lower or higher prey densities, different anemone species, patchily-distributed prey), switching behaviour would have been observed. The experimental system, however, was not ecologically unrealistic; A.papillosa, found among the mussel beds at Kinkell Braes, would encounter a similar choice between A.equina attached to mussels and S.troglodytes either buried in sand between mussels or exposed like A.equina. Furthermore, the density of prey presented was similar to patches found in the natural habitat. Thus, these results reflect a genuine pattern of behaviour, and switching behaviour as an adaptive mechanism to improve foraging efficiency, at the prey relative abundances used in this experiment, is not demonstrable. It should perhaps be noted that the results obtained in this study are not dissimilar to those of Murdoch (1969) in his studies on Acantina spirata.

It is possible that at more extreme ratios of prey abundance the conditioning effect, through feeding in particular habitat patches, is sufficient to effect switching behaviour. In designing the experiment more extreme ratios were expected to be less informative than 7:3 in defining the shape of a switching curve, should such be demonstrable. In retrospect perhaps an 8:2 ratio would have proven more valuable.

The results of the present experiments do show that the ingestive conditioning phenomenon demonstrated in chapter 5 does operate, at least for S.troglodytes, in this system where the consumption of intact anemones is required and where the conditioning diet is only relatively more abundant than an alternative prey. The absence of any apparent conditioning to A.equina remains somewhat enigmatic. The escape responses described previously for A.equina may be important in this respect. Such defensive adaptations may make it advantageous (at the densities used in this experiment) for A.papillosa to prey on S.troglodytes regardless of relative abundance. The degree to which such conditioning changes the observed behaviour of the nudibranch is an indication of the effectiveness of this phenomenon under more biologically realistic circumstances. Thus, the results indicate that the overall preference for S.troglodytes that was demonstrated by five of the six nudibranchs in the experiment could not be reversed by circumstances that might be expected to prevail in the natural habitat.

The reasons for the preference shown for S.troglodytes are unclear, and the analysis of prey-values in terms of growth and reproduction shed little light on this problem.

As discussed previously, however, S.troglodytes stands out among the species studied as being somewhat different from the others. This anemone appears to be assimilated more efficiently and is indicated as possibly being consumed at a faster rate. It should be emphasised, however, that it has the lowest calorific value of all the anemones studied (Table 3) and did not result in improved growth or reproduction (Figs. 2 and 5). Anemone defenses, as determinants of prey selection by A.papillosa, may, in fact, play a key role in the dynamics of this system.

In the present context it is relevant to consider the apparently frequent and extensive association that A.papillosa demonstrates in the intertidal with S.troglodytes. Large numbers of A.papillosa have been found in such association and many, indeed, have been observed spawning. This indicates that A.papillosa metamorphosing among extensive buried S.troglodytes populations will successfully survive, grow and reproduce in such circumstances. Under these conditions, however, individual adult nudibranch sizes were invariably small (the majority of A.papillosa collected in association with S.troglodytes during May and June, 1983 were between 1.0 and 2.0g) and markedly below maximum attainable sizes (the largest A.papillosa in laboratory studies exceeded 11.0g).

Small adult sizes here will be the result of a combination of at least three important ecological factors:

i) S.troglodytes is a relatively small anemone and may, therefore, constitute a sub-optimal prey item for large A.papillosa.

ii) The ability of S.troglodytes to withdraw into sand (and, perhaps, a crevice in the hard substratum to which it is attached or between Mussels) may afford partial or total protection from a large predator.

iii) Large nudibranchs associated with, for example, the extensive S.troglodytes beds at Robin Hood's Bay (see Plate 3), would not survive wave crash or wave surge in this exposed habitat due to the scarcity of physically protective microhabitats.

S.troglodytes may, therefore, by virtue of its small size, abundance and extensive distribution on rocky shores around the British Isles, comprise an ideal associate for juvenile A.papillosa.

Extended survivorship and larger (to maximal) body sizes among S.troglodytes associates would, for the reasons outlined above, be unlikely in the field: hence, fitness would, perhaps, be maximal for individuals of A.papillosa associated with, for

Plate 3.

The intertidal habitat at Robin Hood's Bay, North Yorkshire, in which smaller A.papillosa are common in association with S.troglodytes. The boulders are both approximately 1m in diameter and the nearest is some 10m distant. The photograph was taken at E.L.W.S. and shows the incomplete drainage of the exposed, flat surface forming small pools among Mytilus clumps and associated sand. The almost total absence of any algae is a further indication of the degree of exposure. Nudibranchs were generally located at the pool margins around the periphery of Mytilus clumps. Mussels were very small (1-2cm maximum size) and dense.

example, the larger A.equina or U.felina in the protected under-stone habitat. It is nonetheless quite likely that juveniles might frequently settle in association with S.troglodytes and subsequently become relocated in the understone habitat thereafter.

Chapter 7.

SUMMARY AND CONCLUSIONS

Optimal foraging models have proved to be of considerable value in the analysis and description of predator behaviour. In the present study, however, specific optimal foraging models have not been constructed or tested. Necessarily, simplistic optimality models do not, in most cases, accurately describe or predict the foraging behaviour of predators. Notable exceptions, however, include, for example, the studies of Belovsky (1978, 1981) and Belovsky & Jordan (1978) on the North American Moose and of Ostfeld (1982) on the Californian sea-otter. Despite these and other exceptions, in which relatively simple models do successfully predict behaviour, it seems that, for most predators, there is a marked element of unpredictability in foraging behaviour. Such 'noise' in observed predatory behaviour is likely to be, at least in part, a consequence of stochastic factors associated with each prey encounter. For example, in the case of A.papillosa, localised fluctuations in water currents may result in a different species of anemone being detected and eaten on different occassions. Furthermore, specific complexities, associated with each predator-prey association may further detract from the accuracy of foraging model predictions. Each animal as a 'special case' may, indeed, be the rule rather than the exception. Despite this, however, such models are of considerable value by virtue of their capacity to focus attention

on possible factors which may be important underlying determinants of foraging behaviour for many species.

In the present study the demonstration of ingestive conditioning for A.papillosa and considerations arising from optimal foraging theory led us to predict that the nudibranch would demonstrate switching behaviour (sensu Murdoch,1969; Murdoch & Oaten,1975; Murdoch et al.,1975). On a theoretical basis such a prediction was entirely reasonable; A.papillosa appears to fulfill many of the assumptions under which switching behaviour should occur. Notably:

i) The nudibranchs prey on more than one sympatric prey species.

ii) A training effect (sensu McNair,1980,1981), at least with regard to the selection of water-borne chemical prey-stimuli, is clearly demonstrable.

iii) Previously published studies had indicated a distinct variability in prey-preference even within restricted localities (e.g. Stehouwer,1952; Braams & Geelen,1953; Robson,1961; Waters,1973; Edmunds et al.,1974,1976).

Despite the apparent suitability of A.papillosa as a 'switching predator', such behaviour could not be demonstrated; in most cases, a marked preference was shown for S.troglodytes. Thus, in the present study, a prediction based on sound and demonstrated principles (see, for example, Lawton et al.,1974;

Bayliss,1982) could not be upheld. Reasons for the observed preference for S.troglodytes are, as yet, undetermined. Within the context of the switching experiment methodology, this may have been a consequence of the simplified environment where no protection in crevices or between mussels was afforded to S.troglodytes. Furthermore, it would appear that the detachment response of A.equina may have notably reduced the frequency with which this species was selected.

'Prey-value', derived in its current biological sense from optimal foraging models (e.g. Pyke et al.,1977; Hughes,1979), is expected to be an important determinant of prey-selection behaviour. In the present study 'prey-values', or more specifically, food or tissue-values (because intact anemones were not used in the analyses), have been investigated for a range of anemone species. Analysis of the biochemical composition of anemone tissues and their consumption and assimilation by nudibranchs did not reveal any marked interspecific differences. S.troglodytes, however, is indicated as being somewhat different from the other species studied, possibly being assimilated more efficiently and consumed at a faster rate. These factors, in conjunction with the strong preferences shown for S.troglodytes in switching experiments and the apparent preponderance of field associations with this species, do indicate that S.troglodytes may be a more valuable food item for A.papillosa. Analysis of the composite estimates of fitness (growth and reproduction), however, did not reveal any contrasts in performance which could be related to diet. This was almost certainly a result of the

marked variability in the performance of nudibranchs within each diet group obscuring any dietary effects which may have obtained.

Although the food or tissue-values of anemone species do not appear to differ markedly from one another, it is not possible to make any statements regarding intact anemones, or the effects of the specific habitat in which they occur. Such factors will undoubtedly be important in determining patterns of predation and association between the nudibranch and its prey. In particular, the size that a prey species normally achieves is likely to be important. It is reasonable to suppose, for example, that large M.senile or U.felina represent low-value prey for small or juvenile A.papillosa in view of the (possibly) higher risk of mortality when attacking such well-defended prey. By contrast, for large adult nudibranchs S.troglodytes may represent a sub-optimal prey species by virtue of its small size or, perhaps more importantly, its occurence in exposed, high energy, intertidal habitats: in such environments large A.papillosa are unlikely to be able to maintain themselves due to wave crash and surge. In addition, one may expect differences in prey-values resulting from species-specific suites of defense and escape adaptations (e.g. the detachment response of A.equina, burial by S.troglodytes or protection afforded by adherent shell fragment for U.felina).

In this study no account has been taken of the planktotrophic larval phase of A.papillosa, although a number of unsuccessful attempts were made to culture the larvae through to metamophosis. The importance of this stage in the life-cycle, with regard to foraging behaviour, is unestablished although it is certain to be of considerable importance. Nudibranch larvae invariably require the presence of the live adult diet before they will metamorphose. In the case of A.papillosa, where a number of prey species are acceptable dietary items, the extent to which specific anemones differentially elicit settlement responses is of critical importance. In the absence of any marked specificity at settlement one would expect most larvae to settle on, and become conditioned to, the most abundant prey species in the habitat. Through subsequent feeding the predator would act to stabilise or reduce the numbers of that prey species in the habitat. If, however, the larvae demonstrate specific preferences at settlement, subsequent patterns of prey selection by the newly-settled nudibranch population, re-inforced by ingestive conditioning, may exert a marked predation pressure on a species that is uncommon. Until the presence or absence of any specificity at settlement is established the dynamics of these aeolid-anemone associations must necessarily remain somewhat speculative.

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